TYPE I RECEPTORS MEDIATE THE ENHANCING EFFECTS OF CORTICOSTERONE UPON CONVULSION SUSCEPTIBILITY IN MICE

by

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A DISSERTATION

Presented to the Department of Medical Psychology and the Graduate Council of the Oregon Health Sciences University School of Medicine

In partial fulfillment of the requirement for the degree of

Doctor of Philosophy

October, 1993

APPROVED

[redacted]

(Professor In Charge of Thesis)

[redacted]

(Chairperson, Graduate Council)

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ACKNOWLEDGEMENTS

The ABC's of my Ph.D.

A is for **A**dvisor, Don has been the best
B is for **B**iochemistry, a class I gladly missed
C is for **C**hris Cunningham, a mentor to us all
D is for **D**ebbie, with whom I've had a ball
E is for **E**laine, an excellent friend and a very cool dude
F is for **F**unding, for books, travel, and especially food
G is for **G**inger, who has helped me when I'm stressed
H is for Greg **H**enkel, whose laughter is the best
I is for **I**deas, discussed with John and Ed
J is for **J**ane, my favorite running friend
K is for **K**im, my wonderful childhood chum
L is for the **L**ab, where most of my work was done
M is for **M**ice: WSP and WSR
N is for **N**ewman and Keuls, whoever they are
O is for **O**ur department, which has a lot of flare
P is for **P**roposals, I certainly wrote my share
Q is for **Q**uestions, turned in weekly for seminar
R is for **R**SA, a meeting spent in a bar
S is for **S**helly, **S**andra, and **S**arah; 3 wonderful gals
T is for **T**alk, to Pam, Jim, Hideki, and my other pals
U is for **U**pbringing, my parents raised me well
V is for the **V**ollum, they can all go run a gel
W is for Cristina **W**eaver, my great college buddy

X is for **X**erox, all the copies that came out cruddy
Y is for **Y**ou, Mark, my favorite person indeed
Z is for the **Z**ombie I was just before I got degree'd.

Don has been an excellent advisor and a great friend. He truly cares about students and has put huge amounts of energy into my training. I am grateful for his guidance over the years.

A lot has changed in my life during my five years in graduate school. I got married, became close friends with my mom and dad, had the opportunity to spend considerable time with my brother and nephews, and ran a couple of marathons. These have been positive influences in my life; I owe so much to my wonderful family!

I dedicate this thesis to my husband, Mark Hansen, a wonderful and supportive friend.

I. ABSTRACT

It is generally acknowledged that corticosteroids act in the periphery to re-establish homeostasis following stressful insults. Recently, however, evidence has accumulated which suggests that these hormones also have profound central actions. Both excitatory and inhibitory effects of corticosteroids upon measures of nervous system excitability have been reported. The outcome of these experiments appears to depend on such factors as the doses and structures (mineralocorticoid versus glucocorticoid) of the corticosteroids used, the animal species tested, the measure of excitability used, and whether the experiments were performed *in vivo* or *in vitro*.

Two corticosteroid receptors exist in the central nervous system which may mediate the excitatory and inhibitory effects of these hormones. Type I receptors are localized in the hippocampus and bind corticosterone and mineralocorticoids with high affinity. Type II receptors are more widespread throughout the nervous system and bind corticosterone with a lower affinity and glucocorticoids with high affinity. A review of the literature reveals a trend toward excitatory effects of low concentrations of corticosterone and mineralocorticoids and inhibitory effects of high concentrations of corticosterone and glucocorticoids. This has led to the hypothesis that type I receptors mediate excitatory and type II receptors mediate

inhibitory effects of corticosteroids on nervous system excitability.

This hypothesis was tested in the present study by examining the effects of corticosteroid manipulations on convulsions produced by three chemicals in genetically heterogeneous mice. Pentylenetetrazol (PTZ), strychnine, and kainic acid were chosen based on their diverse mechanisms of action. PTZ acts on the gamma-aminobutyric acid (GABA) receptor complex and produces convulsions which are believed to involve several brain regions. Strychnine acts on the glycine receptor and produces convulsions believed to be of spinal cord and brainstem origin. Kainic acid acts on a subset of glutamate receptors and is believed to produce limbic convulsions.

It was hypothesized that corticosterone, via activation of type I receptors, would decrease doses of the convulsants required for convulsions and that corticosterone, via activation of type II receptors, would increase doses of the convulsants required for convulsions. An examination of the effects of corticosteroid manipulations on multiple convulsion signs made it possible to determine whether corticosteroid effects on nervous system excitability were global or whether they displayed neuroanatomical and/or pharmacological specificity.

The results of these experiments supported a role for type I receptors in mediating excitatory effects of corticosteroids upon convulsions. Interestingly, only

certain convulsions were sensitive to manipulations of type I binding. These convulsions were PTZ-induced myoclonic jerk, face and forelimb clonus, and tonic hindlimb extension, and kainic acid-induced wild running clonus and tonic hindlimb extension. The limbic system has been implicated as the site of origin for these convulsions (except PTZ-induced tonic hindlimb extension), therefore it is possible that corticosteroids, via type I receptor activation, enhance hippocampal excitability.

No inhibitory effects of corticosterone were observed in the present experiments. Therefore, it was not possible to test the role of type II receptors in mediating inhibitory corticosteroid effects. However, the type II antagonist RU38486 displayed proconvulsant action over convulsions produced by PTZ and kainic acid. Although this is consistent with an inhibitory role of type II receptors, it is not clear whether the effect of RU38486 was truly due to type II receptor antagonism or some other effect of this drug.

These data add to the growing body of work supporting a balance in type I- and type II- mediated responses to corticosteroids being critical for the setpoint of homeostatic control over central nervous system excitability. Furthermore, these data may have clinical relevance, as type I corticosteroid receptor antagonists may be useful anticonvulsants for use in particular epilepsies.

II. INTRODUCTION

II.A. Corticosteroids

II.A.1. *General background*

The hormones of the hypothalamic-pituitary-adrenocortical (stress) axis allow organisms to respond to and adapt to stressors. A stressor is defined as any event that disrupts homeostasis (Johnson, Kamilaris, Chrousos and Gold, 1992). Examples of stressors include disturbances in the internal environment (eg. hypoglycemia), disturbances in the external environment (eg. extreme cold), pain and injury, food delivery, fear, anxiety, joy and frustration. Stress is the nonspecific response of the body to such stressors. Operationally, stress is generally defined as a state of increased activity of the hypothalamic-pituitary-adrenocortical axis.

In response to a stressor, corticotropin-releasing factor, arginine vasopressin, and perhaps other factors, are released from the hypothalamus into the portal blood stream. These stimulate the secretion of adrenocorticotropin (ACTH) from the anterior pituitary into the general circulation. ACTH, in turn, stimulates the synthesis of corticosteroids in the adrenal cortex. The activity of the stress axis is under tight negative control by corticosteroids. Therefore, as corticosteroid levels increase, release of corticotropin-releasing factor and ACTH are inhibited. Thus, the stress axis is a positive feed-forward and negative feedback loop.

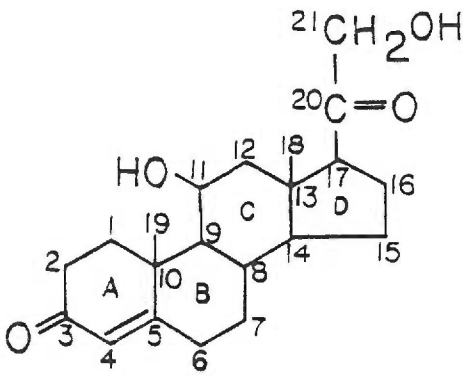
The hypothalamic-pituitary-adrenocortical axis is subject to circadian input from the suprachiasmatic nuclei (Cascio, Shinsako and Dallman, 1987). Circulating levels of ACTH and corticosteroids exhibit an endogenous rhythm of 24 hr. In nocturnal animals, peak hormone levels are observed at lights out and trough levels at lights on. In humans and other diurnal animals, peak hormone levels occur in the morning and trough levels occur in the evening.

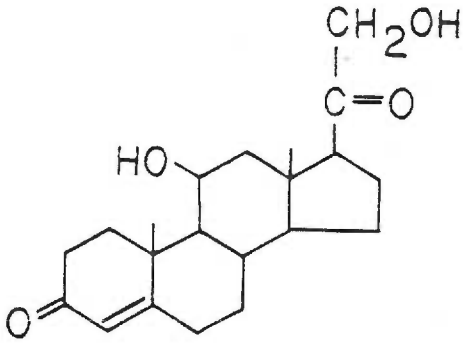
Corticosteroids are typically classified as either mineralocorticoids or glucocorticoids depending on their structures and actions. In humans, the principal mineralocorticoid is aldosterone. Although aldosterone levels are often increased following exposure to stressors, this hormone is primarily regulated by the renin-angiotensin system. The principal glucocorticoid in humans is cortisol. This hormone is under primary control of the stress axis. Corticosterone, the principal corticosteroid of rodents, has both mineralocorticoid and glucocorticoid activity. These three hormones are depicted in Figure 1a. Corticosterone is shown twice in order for the ring lettering and carbon numbering sequence to be shown.

Corticosteroid activity requires that the steroid possess 21 carbon atoms, a $-CO\cdot CH_2OH$ side-chain attached at C-17, and a 4-en-3-oxo configuration in ring A (Gower, 1979). Glucocorticoid activity requires an oxygen function at C-11. The presence of a hydroxyl group at C-17 enhances glucocorticoid activity. Mineralocorticoid activity

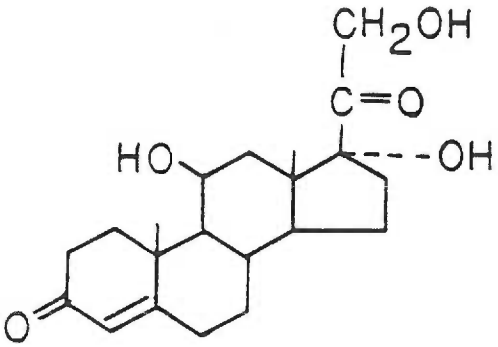
Figure 1. Structures of endogenous corticosteroid (a) and synthetic corticosteroid receptor angonists (b).

a

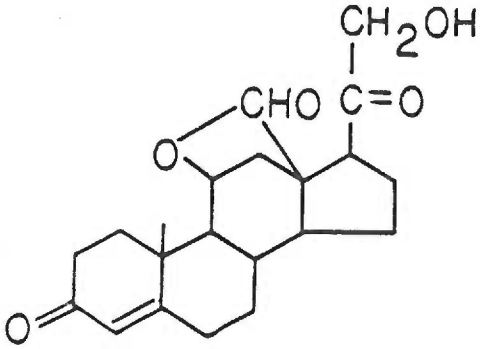




Corticosterone

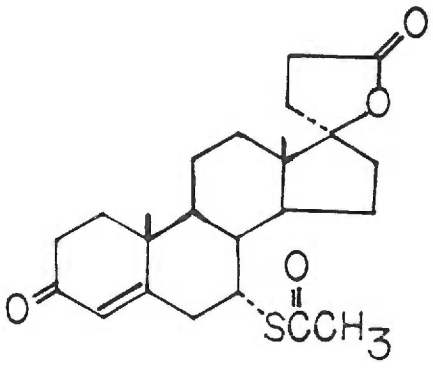


Cortisol

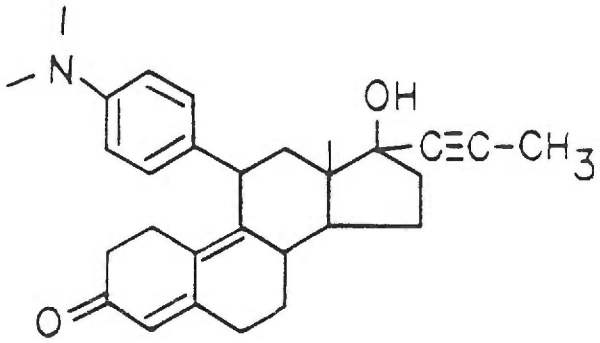


Aldosterone

b



Spironolactone



RU38486

requires a hydroxyl group at C-21. Aldosterone possesses structural features of both a glucocorticoid (11β-hydroxyl) and a mineralocorticoid (21-hydroxyl). Because of their proximity, the 11β-hydroxyl and 18-aldehyde groups interact, producing the hemi-acetal form of aldosterone. This results in a masking of the glucocorticoid activity.

Corticosteroids are inactivated in a NADPH-dependent fashion hepatically or extrahepatically by reduction of the 4-5 double bond (Lindberg, Shire, Doering, Kessler and Clayton, 1972). In the liver, the ketone at position 3 is reduced to a hydroxyl group. This hydroxyl is then coupled with sulphate or glucuronic acid to produce water soluble sulphate esters or glucuronides for excretion. Some reversible oxidation also occurs at position 11. The half-life of corticosterone in the plasma of rats has been reported as 10-20 minutes (Glenister and Yates, 1961; Kitay, 1961; Dallman and Yates, 1969; Harris and Reynolds, 1979).

Corticosteroids have many actions in the periphery that are involved in the re-establishment of homeostasis following a stressful insult (Selye, 1946). They are involved in releasing energy substrates such as glucose, fatty acids, and amino acids from storage sites to the bloodstream and stimulating cardiovascular and pulmonary function. Mineralocorticoids are involved in sodium retention and therefore regulate the balance of electrolytes and water in many tissues of the body. Glucocorticoids aid

liver glycogen deposition, exert "permissive" and also perhaps "regulatory" control over carbohydrate, protein, and lipid metabolism, and suppress immunological and inflammatory responses (Baxter and Tyrrell, 1981). A current theory which takes into account the inhibitory effects of glucocorticoids on many systems, proposes that these hormones maintain homeostasis by turning off defense reactions activated by stressors before they overshoot (Munck, Guyre and Holbrook, 1984).

In addition to their classical peripheral effects, corticosteroids have been shown to have profound central effects. The literature is confusing, as both excitatory and inhibitory effects of corticosteroids have been reported. This confusion is probably due to several factors such as the various corticosteroids used in the studies, the range of doses used, the species investigated, whether experiments were performed *in vivo* or *in vitro*, and the measure of excitability used. This literature will be reviewed briefly below. First, however, the two corticosteroid receptors located in brain will be discussed, as these potentially mediate excitatory and inhibitory effects of these steroids.

II.A.2. *Corticosteroid receptors*

There are two classes of cytoplasmic receptors specific for corticosteroids in the central nervous system which may mediate steroid effects on neural excitability (Funder and

Sheppard, 1987). Table 1 shows general characteristics of these receptors which will be discussed below. Type I and type II corticosteroid receptors belong to the steroid receptor super family which includes receptors for androgen, estrogen, progesterone, and vitamin D. These cytoplasmic receptors are inactive in the absence of specific ligands. Addition of hormone results in rapid transformation of these receptors to their active state. It has been proposed that the inactive state is maintained by association of the receptor with other proteins (reviewed by Carson-Jurica, Schrader, O'Malley, 1990). A heat shock protein (HSP 90) has been found in complexes with type I and type II receptors. In the presence of HSP 90, steroid receptors are unable to bind DNA. Hormone treatment results in dissociation of the complex and a receptor that can be activated. A second step in activation may involve phosphorylation of the receptor. Activation results in a conformational change in the receptor protein allowing it to translocate to the nucleus.

The receptor domain responsible for DNA binding has received considerable attention (O'Malley and Tsai, 1992). It is a cysteine-rich region capable of binding zinc in a manner that creates two peptide projections referred to as zinc fingers. These zinc fingers are important for interactions of receptors with target gene enhancer regions. Target DNA sequences for receptor interaction are termed steroid response elements (SRE). In general, SRE's are 15-

Table 1. Characteristics of type I and type II corticosteroid receptors. (See text for references)

	Type I	Type II
Affinity for corticosterone	0.5 nM	5 nM
Location in central nervous system	Primarily in hippocampus	Widespread throughout brain
Agonists	Corticosterone, Cortisol, Deoxycorticosterone, and Aldosterone	Corticosterone, Cortisol, and Dexamethasone
Antagonists	Spironolactone and RU26752	RU38486
Function	State of arousal?	Negative feedback upon higher levels of the stress axis

base pair consensus sequences, composed of two half-sites of six base pairs arranged in a dyad axis of symmetry around a few base pairs of random composition. Steroid receptor dimers bind with a greater affinity and stability to their SRE's than receptor monomers (ibid). This binding of SRE's in the enhancer regions of genes appears to stabilize transcription factor associations with promotor regions, thus enhancing transcription.

Type I receptors are localized primarily in the hippocampus, most densely in the dorsal subiculum and CA1 field, but also abundantly in the dentate gyrus and CA2 and CA3 cell fields. Type I receptors bind corticosterone with relatively high affinity (0.5 nM; Reul and de Kloet, 1985; Reul, van den Bosch and de Kloet, 1987) and are believed to be fully occupied during the circadian peak. These receptors are also referred to as mineralocorticoid receptors because of their similarity to the classic renal mineralocorticoid receptors in their affinities for natural and synthetic steroids (Krozowski and Funder, 1983). They bind aldosterone and deoxycorticosterone, but have much lower affinity for the synthetic glucocorticoid, dexamethasone. Type I receptors are believed to mediate the tonic influence of corticosterone on cognition, mood, and affect (de Kloet and Reul, 1987), sensory interpretation and sleep regulation (Reul, van den Bosch and de Kloet, 1987), and circadian regulation of ACTH secretion (Dallman, Levin, Akana, Jacobson and Kuhn, 1989).

Spironolactone, ZK91587, and RU26752 are currently available type I antagonists with high affinity (approximately the same Kd as corticosterone) and specificity (Lazar and Agarwal, 1986; Sutanto and de Kloet, 1988). Preliminary experiments in our laboratory using RU26752 have shown promise; however, because of its limited availability and scarce background literature, the more commonly used antimineralocorticoid, spironolactone will be used in the present experiments. The structure of spironolactone is shown in Figure 1b.

Type II receptors are widespread throughout the brain and periphery. For example, they are located in the anterior pituitary, median eminence, paraventricular nucleus, solitary nucleus, supraoptic nucleus, amygdala, locus coeruleus, raphe area, and in lower abundance in the hippocampus. These receptors are also referred to as glucocorticoid receptors because of their similarity to the classic peripheral glucocorticoid receptors in their affinities for natural and synthetic steroids. Type II receptors bind corticosterone with a lower affinity (5 nM) relative to type I receptors and are only fully occupied during exposure to stressors. These receptors have high affinity for dexamethasone and much lower affinity for mineralocorticoids such as aldosterone and deoxycorticosterone. The principal role of these receptors appears to be the mediation of the feedback action of corticosterone on the pituitary to inhibit stressor-induced

ACTH synthesis (Reul and de Kloet, 1985; Reul, van den Bosch and de Kloet, 1987; Ratka, Sutanto, Bloemers and de Kloet, 1989). These receptors have also been implicated in the circadian regulation of ACTH secretion (Reul and de Kloet, 1985).

RU38486,a synthetic antagonist at type II glucocorticoid sites with a Kd of approximately 0.5 nM (Dayanithi and Antoni, 1989), will be used in the present experiments. Its structure is shown in Figure 1b. This drug (a.k.a. RU486 or mifepristone) also antagonizes progesterone binding to its receptor. This property of the drug has been the focus of controversy because of its ability to terminate early pregnancy (Ulmann, Teutsch and Philibert, 1990). RU38486 has been reported to increase K+ uptake in cultured cortical neurons (Bauer and Bauer, 1990). Whether this action was via antagonism of progesterone or glucocorticoid at their receptors was not conclusively determined. This drug has been shown to block the inhibitory action of dexamethasone (Allen, Sutanto and Jones, 1988) and corticosterone (Dayanithi and Antoni, 1989) on corticotropin-releasing factor-stimulated ACTH secretion *in vitro*. Preliminary evidence suggests that RU38486 may be useful in patients suffering from Cushing's syndrome (hypercortisolemia) in lieu of adrenalectomy as it inhibits cortisol action at its receptor (Beaufrere, De Parscau, Chatelain, Morel, Aguercif and Francois, 1987; Laue,

Chrousos, Loriaux, Barnes, Munson, Nieman and Schaison, 1988).

The binding characteristics of some steroid receptors differ depending on what tissue is being studied and whether binding studies are performed *in vivo* or *in vitro* (Roy, 1992). For example, *in vitro* studies of the rat type I mineralocorticoid receptor have shown that its affinity for aldosterone is equal to its affinity for corticosterone. However, when examined *in vivo*, type I receptors in the kidney, gut, and parotid displayed selective binding for aldosterone, whereas type I receptors in the hippocampus also bound corticosterone. It has been suggested that the enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSD) is responsible for determining the binding characteristics of the type I receptor (Funder, Pearce, Smith and Smith, 1988; Edwards, Burt and Stewart, 1989). This enzyme is responsible for the inactivation of cortisol (by oxidation) and corticosterone (by reduction). The high levels of 11β-OHSD in the kidney, gut, and parotid protect the type I receptor from cortisol and corticosterone. In the hippocampus, however, 11β OHSD levels are very low, therefore cortisol and corticosterone are available to be bound by type I receptors.

It is not fully understood at present exactly how steroid receptor antagonists inhibit steroid action. Steroid receptor antagonists may inhibit steroid action at one or more steps in the receptor cascade (activation,

translocation, DNA interaction). Most of the experiments investigating this issue have made use of the type II corticosteroid receptor antagonist, RU38486 (reviewed by Mao, Regelson and Kalimi, 1992). RU38486 has been shown to be bound in a specific manner by type II receptors. Preliminary evidence suggests that it binds to the same receptor site as the agonists; however, it appears to display different binding characteristics. RU38486 binding was found to decrease the amount of glucocorticoid receptor present in its lower density form, suggesting that it inhibits the dissociation of HSP 90 from the receptor (Beck, Estes, Bona, Muro-Cacho, Nordeen and Edwards, 1993). There is, however, evidence of some degree of activation of antagonist-receptor complexes. These complexes may be altered in such a fashion that they are unable to interact properly with nuclear pore elements resulting in a failure to translocate to the nucleus. Alternatively (or in addition), activated antihormone-receptor complexes may bind SRE's in a different manner than activated hormone-receptor complexes. The conformational changes in the receptor induced by RU38486 were found to be different from conformational changes induced by agonist (Beck et al., 1993). Other corticosteroid receptor antagonists may inhibit hormone action at the same or different steps in the receptor cascade.

Many genes are under the control of steroids; however, the roles of the gene products in mediating corticosteroid

effects on nervous system excitability are unknown. It has been shown that aldosterone via peripheral type I receptors increases the transcription of specific mRNA encoding citrate synthase and the α and β subunits of the Na^+, K^+-ATPase (Johnson, 1992). In the rat hippocampus, it has been shown that corticosterone increases the amount of protein 1, a nervous system-specific phosphoprotein (Nestler, Rainbow, McEwen and Greengard, 1981). Although the precise role of this protein is unknown, it is localized to presynaptic terminals of neurons and is associated with neurotransmitter vesicles. This suggests that protein 1 may play a role in modulating neurotransmitter release. Protein 1 levels were unaffected by dexamethasone, suggesting that the effect of corticosterone on its synthesis was via type I receptors. Several mRNAs have been cloned from rat hippocampus which are responsive to corticosterone administration (Nichols, Masters, May, Vellis and Finch, 1989). These were subsequently found to be differentially responsive to vibratory stress (Nichols, Masters and Finch, 1990). Levels of only one of the three clones were increased by stressor exposure. It appeared that the temporal aspects of corticosterone regulation may account for this finding.

Corticosteroids have been shown to alter many neurotransmitter systems including the serotonergic, β-adrenergic, and dopaminergic systems (reviewed by Hall, 1982; Anisman and Zacharko, 1990). For example, low doses of corticosterone (1-2 mg/kg) were found to increase rat

brain serotonin levels, whereas a high dose (10 mg/kg) decreased levels of this neurotransmitter (Kovács, Telegady and Lissák, 1975). Acute corticosterone (15 mg/kg) significantly increased norepinephrine synthesis in whole mouse brain (Iuvone, Morasco and Dunn, 1977); however, acute cortisol (10 mg/kg) decreased brain levels of norepinephrine in rats (Laborit and Thuret, 1977). These and other discrepant findings may reflect a biphasic dose-response phenomenon for the effects of corticosteroids on brain norepinephrine levels. Restraint-stress was found to enhance dopamine release in the nucleus accumbens and prefrontal cortex of rats using *in vivo* microdialysis (Imperato, Puglisi-Allegra, Casolini, Zocchi and Angelucci, 1989). In this study, it was also shown that this stressor increased the release of acetylcholine in the hippocampus. These effects were reduced by adrenalectomy and mimicked in non-stressed rats with administration of 3 mg/kg corticosterone. Repeated restraint-stress was shown to alter dopamine receptor densities in nucleus accumbens and caudate-putamen (Puglisi-Allegra, Kempf, Schleef and Cabib, 1991). Although these effects appear to be corticosterone-dependent, it is not clear whether they result from the action of this hormone at type I and/or type II corticosteroid receptors.

It is becoming increasingly clear that corticosteroids exert some effects which are independent of binding to their classical cytoplasmic receptors. Evidence is accumulating

which suggests that steroids may interact directly with neurotransmitter receptors. For example, steroid interaction with the $GABA_A$ receptor complex is currently receiving considerable attention. This receptor complex will be discussed in the next section in relation to the chemical convulsant, pentylenetetrazol. In general, it appears that low-to-moderate concentrations of corticosteroids inhibit chloride influx and high concentrations enhance chloride influx through the GABA ionophore (Majewska, 1987a, 1987b; Majewska, Bisserbe and Eskay, 1985). Several steroid hormone metabolites have been shown to be bound by the GABA receptor complex (Puia, Santi, Pritchett, Purdy, Paul, Seeburg and Costa, 1990). Therefore, it is possible that some effects of corticosteroids on nervous system excitability may be mediated by direct modulation of GABAergic neurotransmission.

II.A.3. *Central action of corticosteroids*

As was mentioned above, there is considerable controversy in the literature concerning the effects of corticosteroids on nervous system excitability. Both excitatory and inhibitory effects of these steroids have been reported. However, the recently recognized receptor heterogeneity may shed light on this controversy. For example, it appears that mineralocorticoids and low concentrations of corticosterone tend to be excitatory,

whereas glucocorticoids and high concentrations of corticosterone tend to be inhibitory. This has led to the hypothesis that type I receptors mediate excitatory and type II receptors mediate inhibitory effects of corticosteroids on central nervous system excitability (Roberts, Chu, Crabbe and Keith, 1991; Joëls and de Kloet, 1992). Reports of corticosteroid effects on central nervous system excitability will be reviewed below.

II.A.3.a. *Effects of exposure to stressors on nervous system excitability*

Stressors appear to have convulsion-enhancing effects in at least some humans. A positive association was found between subjective stress and seizure frequency in adult epileptics (Temkin and Davis, 1984; Lambie, Stanaway and Johnson, 1986; Webster and Mawer, 1989). In another study, it was shown that certain types of stressors such as worry, frustration, anxiety, and anger were associated with increased seizure frequency in epileptics (Mattson, 1991). A few of these patients were examined closely and it was found that emotional stress led to missed medication, sleep disturbances, or hyperventilation which may have accounted for the increased seizure frequency reported. However, these secondary symptoms of emotional stress are themselves potential stressors. Furthermore, degree of subjective stress has also been shown to be a significant predictor of

psychopathology in epileptics (Hermann, Whitman, Wyler, Anton and Vanderzwagg, 1990).

Stressors have been found to modulate convulsions in laboratory animals. Several studies report seizure-enhancing effects of stressful insults. Seizures were triggered in Mongolian gerbils by exposure to a novel environment; and in several cases these seizures were exacerbated by the extra stimulation of swinging by the tail (Kaplan, 1975). Another study revealed a proconvulsant effect of handling stress (Cain and Corcoran, 1985). Other studies have reported attenuating effects of stressors on convulsion susceptibility. For example, defeat in a social conflict test weakened convulsions observed in fully amygdala-kindled male rats (Beldhuis, Koolhaas and Bohus, 1992). In another study, water immersion stress was associated with decreased severity of pentylenetetrazol-induced convulsions in rats (Abel and Berman, 1993).

The inconsistency in these data may reflect the complexity of the stress response. In addition to increased corticosteroid release, changes in other systems such as the opioid, gamma-aminobutyric acid (GABA)ergic, and adrenergic systems occur following exposure to a stressor (Johnson, Kamilaris, Chrousos and Gold, 1992). The relative roles of these systems may vary depending on species and the type of stressor used. Perhaps to avoid this potential complexity, most of the work in this field has focused on the effects of

exogenously administered stress hormones on nervous system excitability.

II.A.3.b. *Effects of exogenously administered corticosteroids on excitability measured in vivo*

Several human case studies suggested that administration of corticosteroids increased seizure susceptibility. Cortisone and ACTH treatment reportedly produced status epilepticus in a small percentage of patients taking these hormones for allergies and connective tissue diseases, respectively (Stephen and Noad, 1951; Dorfman, Apter, Smull, Bergenstal and Richter, 1951). Several of these patients were described as having marked rounding of their faces during hormone treatment. This is a common feature of abnormally high plasma corticosteroid levels.

The animal literature investigating corticosteroid effects on nervous system excitability *in vivo* is larger and more diverse. Several studies have reported excitatory effects of corticosteroid administration. Woodbury (1952) measured thresholds to electroshock-induced tonic hindlimb extensor convulsions in rats injected daily with various steroids. Cortisol (10 mg/kg) significantly decreased seizure thresholds, suggesting it had proconvulsant action. Corticosterone (10 mg/kg) had a moderate proconvulsant

effect on the first few days of administration, but had no effect subsequently. It was unclear from this report how long following steroid administration electroshock thresholds were determined. It was also unclear whether the steroid administration procedure produced physiologically relevant cortisol and corticosterone levels as plasma concentrations were not determined.

Heuser and Eidelberg (1961) showed that administration of high doses of a precursor of cortisol to rats and cats produced tonic-clonic convulsions which began after 15-35 minutes and reappeared repeatedly until the animals died or were treated with a general anesthetic. In the same study, electroencephalography (EEG) was employed in freely behaving cats following steroid administration. Increased excitation was first detected in the amygdala, then paroxysmal spiking occurred in the hippocampus, followed by spiking in other brain areas, and finally generalized high frequency firing accompanied the development of grand mal convulsions. Plasma cortisol levels were not determined, therefore it is unclear whether the large injection of steroid presursor produced physiological or pharmacological cortisol levels.

In another study, peripheral administration of cortisol (1-4 mg/kg) increased the rate of firing of units in the hypothalamus, reticular formation, and to a greater degree the hippocampus in freely behaving rats (Dafny, Phillips, Newman Taylor and Gilman, 1973). Despite the fact that cortisol is not a naturally occurring steroid in the rat,

plasma levels were in the range normally observed in response to a variety of stressors.

In the snail, dexamethasone produced a slight decrease in neuronal firing, followed by a larger excitatory effect (Schadé and Wilgenburg, 1973). More recently, an excitatory effect of dexamethasone was found in rodents. It was shown that dexamethasone (0.5 and 1.0 mg/kg) increased the number of kainic acid-induced wet dog shakes in rats, but had no effect on latencies to, or number of, kainic acid-induced motor seizures (Lee, Grimes and Hong, 1989). Because dexamethasone is not bound in plasma by corticosterone-binding globulin, free levels of this steroid would be expected to be much higher than those following equivalent doses of corticosterone or cortisol. It is not clear if levels of dexamethasone achieved were of physiological relevance.

In several studies, excitatory effects of corticosteroids applied directly to brain regions were found. In immobilized cats, cortisol increased the amplitude of evoked potentials recorded from the hypothalamus, thalamus, and reticular formation (Feldman, Todt and Porter, 1961). ACTH had similar effects which took longer to develop, presumably because its effects were due to secondary increases in endogenous corticosteroid levels. Microelectrophoretically- applied corticosterone increased the firing rates of 61% of Raphe neurons recorded from in

anesthetized rats (Avanzino, Ermirio, Ruggeri and Cogo, 1984). The other 39% were unresponsive to corticosterone.

Excitatory effects of corticosteroids upon drug (especially ethanol) withdrawal convulsions have been widely observed. Withdrawing alcoholics have been shown to have enhanced hypothalamic-pituitary-adrenocortical axis activity, perhaps accounting for some of the signs of withdrawal (Muller, Hoehe, Klein, Nieberle, Kapfhammer, May and Muller, 1989; Adinoff, Risher-Flowers, De Jong, Ravitz, Bone and Nutt, 1991). Indeed, withdrawal symptoms were often reportedly more severe in patients experiencing greater stress axis disturbance.

Ethanol withdrawal has been associated with increased plasma corticosterone levels in mice. Male AKR/J mice were exposed to ethanol vapor for 72 hours followed by hourly handling-induced convulsion scoring (Keith, Crabbe, Robertson and Young, 1983). The peak blood ethanol concentration obtained was 2.34 mg/ml. Tail blood was sampled for corticosterone determinations at either 0, 6, 12, or 24 hours following removal from the vapor chambers. Corticosterone levels were elevated significantly at the time of withdrawal (hour 0) and showed further elevations at 6 and 12 hours, before returning to basal levels by 24 hours post withdrawal. Using the same paradigm, it was shown that handling-induced convulsion scores were elevated at 6 and 12 hours following ethanol withdrawal (Crabbe, Keith, Kosobud and Stack, 1983). In another study, C57BL/6 mice were fed

on a liquid diet containing ethanol for 8 days (Tabakoff, Jaffe and Ritzmann, 1978). Individual ethanol intake was about 26 g/kg/day and the peak blood ethanol concentration obtained was 2.53 mg/ml. Following ethanol withdrawal, circulating corticosterone concentrations increased and peaked at 13 hours, corresponding to the time of peak in behavioral symptoms of withdrawal and withdrawal hypothermia (Ritzmann and Tabakoff, 1976).

The pioneering animal research investigating the relationship between the stress axis and ethanol withdrawal was done by Sze and colleagues (Sze, Yanai and Ginsburg, 1974; Sze, 1977). Male CD1 and C57BL/10Bg inbred mice were fed liquid diets containing ethanol or control diets for 21 or 14 days, respectively. Half of the animals per group had been adrenalectomized 14 days prior to the initiation of ethanol treatment. The average daily intake of ethanol for the ethanol groups was 340-440 mg/mouse/day (approximately 10-15 g/kg/day) with a peak blood ethanol concentration of 3 mg/ml. On the withdrawal day, the control diet was substituted and the incidence of audiogenic seizures was recorded after 7 hours. None of the control mice seized, however 40% (CD1) and 61% (C57) of the intact ethanol-fed mice seized. The occurrence of audiogenic seizures during withdrawal in adrenalectomized mice was only 16%(CD1) and 14% (C57). When daily injections of corticosterone (0.5 mg/mouse) were given to ethanol-fed adrenalectomized CD1 mice, the occurrence of withdrawal seizures was 47%. These

data suggest that a corticosteroid-dependent mechanism is important in chronic ethanol withdrawal seizure expression. This experiment may be criticized on the basis that adrenalectomized mice are so fragile that it is surprising that they survived ethanol treatment. However, this study was important in that it renewed interest in the relationship between corticosteroids and ethanol withdrawal convulsions.

A genetic relationship appears to exist between ethanol withdrawal convulsion severity and stress axis response to ethanol withdrawal or sensitivity to stress hormones. For example, DBA mice displayed more severe acute ethanol withdrawal convulsions and had higher plasma corticosterone levels during withdrawal than C57 mice (Roberts, Crabbe and Keith, 1992). If ethanol withdrawal plasma corticosterone levels were mimicked by injecting ethanol-naive DBA mice with corticosterone, increases in handling-induced convulsions in the range observed during withdrawal were observed.

Withdrawal Seizure Prone (WSP) mice, selectively bred for severe handling-induced convulsions following ethanol withdrawal, were shown to be more sensitive to the excitatory effects of corticosterone than their Withdrawal Seizure Resistant (WSR) counterparts (Roberts, Chu, Crabbe and Keith, 1991). Corticosterone, administered several hours after ethanol, enhanced acute withdrawal handling-induced convulsions of WSP, but not WSR mice. Furthermore,

withdrawal convulsion scores of WSP mice were attenuated by the steroid synthesis inhibitor, aminoglutethimide. Handling-induced convulsions of WSP mice following acute pentobarbital and diazepam (withdrawal precipitated by RO15-1788) were also enhanced by corticosterone, whereas drug-naive convulsions were unaffected (Roberts, Crabbe and Keith, in press (a)).

Inhibitory effects of corticosteroids on nervous system excitability measured *in vivo* have also been reported. Pfaff, Silva and Weiss (1971), showed that peripheral administration of corticosterone decreased hippocampal unit activity recorded from freely moving female rats. The authors administered 0.5 or 1 mg corticosterone intraperitoneally per rat, but did not report body weights or plasma corticosterone levels, therefore it was difficult to determine how physiologically relevant these experiments were.

Inhibitory effects of dexamethasone, a synthetic glucocorticoid, upon nervous system excitability have been documented. Ruf and Steiner (1967) showed that dexamethasone, administered by microelectrophoresis, instantaneously and completely suppressed the firing of about 10% of rat hypothalamic neurons examined *in vivo*. This treatment had no effect on neurons of the cortex, hippocampus, or thalamus. However, Michal (1974) showed that locally applied dexamethasone inhibited the firing rate of rat hippocampal cells *in vivo*. Indeed, dexamethasone was

used successfully in a clinical trial to treat withdrawing alcoholics experiencing delirium tremens (Fischer, Simpson, Smith and Mattox, 1988).

Both excitatory and inhibitory effects of corticosteroids upon *in vivo* measures of nervous system excitability have been reported in animals. Corticosterone and cortisol, while acting primarily as glucocorticoids in the periphery, have been shown to be bound by type I mineralocorticoid receptors in the brain. This tissue specificity is believed to be due to the presence of the inactivating enzyme 11β-OHSD in peripheral tissues, but not in brain (discussed above). Thus, at low concentrations, these hormones would be expected to be bound by type I receptors, while high concentrations would be associated with binding by both type I and type II corticosteroid receptors. Dexamethasone, on the other hand, has very low affinity for type I receptors and is therefore considered the quintessential glucocorticoid.

Most of the experiments mentioned above were not designed to investigate type I- versus type II-mediated corticosteroid effects, therefore steroid doses were often non-physiological and plasma levels were often not reported. In general, there were more reports of excitatory effects of corticosterone and cortisol and more reports of inhibitory effects of dexamethasone.

II.A.3.c. *Effects of decreasing endogenous corticosteroid levels on excitability in vivo*

Both excitatory and inhibitory effects of lowering endogenous corticosteroid levels have been reported. Adrenalectomy is the classical technique used by endocrinologists to decrease circulating corticosteroid levels. Glaser (1953) reviewed reports of the early 1950's and before and suggested that the effects of corticosteroids on seizure state may be due to changes in neuronal permeability and shifts in intra- and extra-cellular electrolytes. It was later shown that adrenalectomy decreased electroshock thresholds and disrupted brain sodium concentrations in rats. If adrenalectomized rats were given 0.9% NaCl to drink, both their electroshock thresholds and their brain sodium concentrations were indistinguishable from intact rats (Timiras, Woodbury and Goodman, 1956). This suggests that mineralocorticoid action may modulate nervous system excitability.

The effects of adrenalectomy on kindled seizures has been investigated. Kindling models an increased state of neural excitability and is used to produce repeatable seizure activity. Kindled hippocampal seizures (both behavioral convulsions and electrical discharges) were decreased by adrenalectomy (Cottrell, Nyakas, de Kloet and Bohus, 1984). Corticosterone substitution reversed the effects of adrenalectomy on these measures.

Aminoglutethimide (Elipten®; α-ethyl-*p*-aminophenyl-glutarimide), a steroid synthesis inhibitor, has also been found to affect convulsion expression in humans and laboratory animals. This drug was originally marketed as an anticonvulsant for use in the treatment of various seizure types (Council on Drugs, 1962). It was removed from the market after it was found that this drug blocked adrenal steroidogenesis by inhibiting the conversion of cholesterol to pregnenolone (Dexter, Fishman, Ney and Liddle, 1967; Fishman, Liddle, Island, Fleischer and Kuchel, 1967; Cash, Brough, Cohen and Satoh, 1967). In our laboratory, aminoglutethimide has been used as a tool for lowering plasma corticosterone levels in lieu of adrenalectomy. Plasma corticosterone levels were significantly decreased in C57BL/6J and DBA/2J mice treated with 17-54 mg/kg aminoglutethimide, while motor performance (rotarod) and body temperature were not altered (Roberts, Gallaher and Keith, in press). Latencies to pentylenetetrazol-induced (145 mg/kg, intraperitoneally) tonic hindlimb extensor convulsions were significantly increased in mice pretreated with 17-54 mg/kg aminoglutethimide (Ahmad and Nicholls, 1990; Roberts, Crabbe and Keith, in press). This effect was found to be reversed by acute corticosterone administration, suggesting that the anticonvulsant effect of aminoglutethimide may be at least partly due to its steroid-inhibiting effect.

As was discussed in the previous section, adrenalectomy and aminoglutethimide treatment resulted in decreased incidence and severity, respectively, of ethanol withdrawal convulsions in mice. In general, decreases in corticosteroid levels appears to be associated with decreased nervous system excitability.

II.A.3.d. *Effects of corticosteroid alterations on excitability measured in vitro*

Experiments performed *in vitro* also support a modulatory role for corticosteroids on nervous system excitability. For example, it was shown that corticosterone, at concentrations which mimicked basal morning and evening states as well as a moderate state of stress, enhanced population amplitudes recorded from rat hippocampal CA1 pyramidal cell fields (Reiheld, Teyler and Vardaris, 1984). In another set of experiments, the effect of adrenalectomy with or without replacement corticosteroids on the after-hyperpolarization (AHP) of CA1 neurons taken from rat hippocampus was examined. AHP occurs following cell firing and is due to the slow inactivation of the K+ conductance which is activated during depolarization. This phenomenon inhibits the transmission of excitatory signals and therefore potentially decreases the excitability of the cell. Hippocampal slices taken from previously adrenalectomized rats were found to display decreased (AHP) amplitudes following a depolarizing current step relative to

tissue obtained from sham-operated control rats (Joëls and de Kloet, 1989). This excitatory effect of adrenalectomy was reversed by corticosterone. In fact, high corticosterone concentrations (1,000 nM) were found to increase AHP amplitudes above those elicited in tissue obtained from sham-operated rats. Low concentrations of corticosterone (1 nM) and the mineralocorticoid aldosterone (1 nM) were shown to decrease AHP amplitudes (Joëls and de Kloet, 1990). Another group examined the effects of low (0.2 nM) and moderate (2-10 nM) concentrations of corticosterone on population spike amplitudes recorded from mouse hippocampal slices (Rey, Carlier and Soumireu-Mourat, 1987). The low dose increased this measure suggesting that it had excitatory effect, whereas the higher doses decreased population spike amplitudes suggesting that they had inhibitory effects.

Long term potentiation (LTP) has been proposed as a substrate for memory as it is characterized by an enhancement in synaptic transmission with rapid onset and long duration. It was shown that the amount of current required to elicit primed burst potentiation, a form of LTP, was lower in hippocampal tissue from adrenalectomized rats than from intact controls (Diamond, Bennett, Engstrom, Fleshner and Rose, 1989). Conversely, primed burst potentiation was completely blocked by stressor exposure (Diamond, Bennett and Rose, 1988). Treatments were administered prior to sacrifice in these experiments,

therefore the concentrations of corticosteroids in the tissues were not controlled.

The literature discussed so far indicates that corticosteroid effects on nervous system excitability *in vivo* and *in vitro* are not clear-cut. Rather, both excitatory and inhibitory effects of corticosteroids (and their removal) have been demonstrated. Many of the studies reviewed above were published before corticosteroid receptor heterogeneity was discovered, therefore experiments were not designed to separate type I and type II corticosteroid receptor effects. Perhaps the clearest existing studies are those employing *in vitro* techniques. In these experiments concentrations of corticosteroids were tightly controlled. In general, low concentrations of corticosterone and mineralocorticoids produced excitatory effects, whereas high concentrations of corticosterone and glucocorticoids produced inhibitory effects on measures of nervous system excitability. This review of the literature has led to the hypothesis that type I receptors mediate excitatory and type II receptors mediate inhibitory corticosteroid effects (Roberts, Chu, Crabbe and Keith, 1991; Joëls and de Kloet, 1992). Further support for this hypothesis will be discussed in the Rationale section.

II.B. Chemical convulsants

II.B.1. *General background*

There are presently a great number of experimental tools available to scientists interested in regulation and control of nervous system excitability. Many of these were mentioned above in relation to central corticosteroid effects. One of the most common models utilizes chemicals which, when administered systemically, produce convulsions. There are several advantages to this tool, including the ease of monitoring the outward convulsion signs, its relative non-invasiveness (does not necessitate surgery and only a single administration is required), and the availability of a wide range of chemical convulsants.

Chemical convulsants have been used predominantly in studies of epilepsy. Epileptic seizures are classified as either partial (focal) or generalized (Fisher, 1989). Partial seizures are subclassified as simple or complex. Simple partial seizures occur without loss of consciousness and may be motor, sensory, sensory-motor, autonomic-visceral, or cognitive in nature. Complex partial seizures result in loss or blunting of awareness and principally involve the temporal (limbic) lobes. Partial seizures can become generalized if there is a spread of epileptiform activity throughout the brain. Generalized seizures involve diffuse and hemispherically symmetrical epileptiform activity and are subclassified as either grand mal or petit mal. Grand mal seizures involve loss of consciousness and

tonic and/or clonic convulsions. Petit mal seizures (also called absence seizures) involve a brief period of staring with or without brief automatisms.

There is considerable controversy regarding the neuroanatomical basis of generalized seizures. A recent model based on animal studies (Gale, 1988), however, suggests that face and forelimb clonus is associated with forebrain circuitry (predominantly limbic), whereas explosive clonus and tonus depend upon brainstem circuitry. Both seizure generating circuits are postulated to be sensitive to modulation by outputs from the midbrain (especially the substantia nigra). This model was developed based on the results of experiments of microinjections of drugs into discrete brain regions and lesion studies.

For example, drug-induced increases in neural transmission in the substantia nigra have anticonvulsant action, while decreased neural transmission increases the likelihood of seizure spread from a variety of brain foci (reviewed by Gale, 1988). The substantia nigra appears to be able to control the propagation of a variety of seizures with different origins and different mechanisms of induction. Microinjections of a variety of convulsants into the "area temptestus" (AT) were found to produce clonic seizures. The AT is localized deep within the prepyriform cortex and is closely associated with limbic circuits. It has been suggested that the AT may represent a site at which agents act to initiate generalized seizures.

Lesion studies support the separation of the forebrain and brainstem as two seizure-generating systems. Stimulation of brainstem regions (particularly the reticular formation) triggers tonic convulsions, and lesions in brainstem and cerebellum selectively attenuate tonic convulsions (Browning, 1987). These manipulations do not alter clonic seizures emanating from the forebrain, however. In addition, precollicular transections, which disconnect forebrain from the rest of the central nervous system, do not prevent tonic convulsions produced by chemoconvulsants.

The neurochemical basis of convulsions is complex and is not fully understood. Normal nervous system excitability is likely maintained by a balance between excitatory and inhibitory events. Seizures may therefore develop in cases of excess excitation or decreased inhibition. It is clear that changes in levels of the inhibitory amino acid neurotransmitter, GABA, can influence seizure susceptibility (Meldrum, 1989; Gale, 1992). In addition, the excitatory amino acid neurotransmitters, aspartate and glutamate (Greenamyre, 1986) and the inhibitory amino acid neurotransmitter, glycine, appear to be important in seizure propagation.

For example, in Gale's model (1988), GABAergic outputs from the pars reticulata of the substantia nigra modulate seizures. The activity of these GABAergic outputs is modulated by neurons releasing the excitatory amino acid neurotransmitters glutamate and aspartate, the neuropeptides

dynorphin, enkephalin, and substance P, as well as GABA itself. The AT contains excitatory amino acid outputs which are modulated by GABAergic and excitatory amino acid inputs. In addition, cholinergic neurons modulate these excitatory amino acid inputs directly and the GABAergic input indirectly through activation of interneurons. In contrast, the inhibitory amino acid neurotransmitter, glycine, is found in abundance in the spinal cord and brainstem (McGeer and McGeer, 1989) and may play an important role in modulating seizure activity in these nervous system regions.

The three convulsants used in the present experiments will be discussed below in relation to what is understood about the seizure types they model, the brain regions they affect, and their possible neurochemical actions. Table 2 reviews the characteristics of these three convulsants.

II.B.2. *Pentylenetetrazol*

PTZ has been used to model generalized seizures in laboratory animals (reviewed by Fisher, 1989; Gale, 1988). Minimal doses administered to laboratory animals result in myoclonus and face (jaw clonus, vibrissae and ear twitching, eye blinking and head nodding) and forelimb clonus. Maximal doses produce running bouncing clonus and tonic flexion and extension of fore- and hindlimbs. EEGs show recurrent runs of rhythmical sharp spiking activity. PTZ is widely used in anticonvulsant screening tests (eg. Krall, Penry, White, Kupferberg and Swinyard, 1978). In addition, it may be

Table 2. Characteristics of PTZ, strychnine and kainic acid. See text for references

Convulsant	Pharmacology	Anatomy	Convulsions
PTZ	Blocks Cl^- influx through the $GABA_A$ receptor complex (decreases Cl^- conductance; depolarizes cells)	Forebrain, hindbrain, and midbrain structures	Myoclonus, face and forelimb clonus, running bouncing clonus, and tonic hindlimb extension
Strychnine	Glycine receptor antagonist (decreases Cl^- conductance; depolarizes cells)	Spinal cord and brainstem	Myoclonus, running bouncing clonus, and tonic hindlimb extension
Kainic acid	Glutamate receptor agonist (increases Na^+ and Ca^+ conductances; depolarizes cells)	Limbic structures	Wild running clonus and tonic hindlimb extension

administered to humans to activate the EEG as a diagnostic aid in epilepsy.

PTZ affects widespread brain areas. Both brainstem and forebrain structures have been implicated as loci for the origin of PTZ-induced seizures (Miller and Ferrendelli, 1988). A comprehensive study by Magistris, Mouradian and Gloor (1988) attempted to establish the respective roles of the forebrain and brainstem in the genesis of PTZ-induced convulsions in the cat. Precollicular transection eliminated clonic convulsions, suggesting that the forebrain is the site of origin for these convulsions. High spinal cord transection in addition to precollicular transection did not eliminate tonic convulsions. This is in support of the view that the caudal brainstem is the site of origin of PTZ-induced tonic convulsions.

PTZ has been shown to interact with the GABA receptor ionophore complex. This complex consists of allosterically linked binding sites for GABA, benzodiazepines, barbiturates, and picrotoxin (Lister and Nutt, 1987). The GABA binding site is coupled to a chloride channel. When GABA binds, the channel opens, chloride ions enter, and the cell membrane hyperpolarizes, thus decreasing the excitability of the cell. Binding of compounds to the other sites serves to modulate chloride influx. Benzodiazepine agonists increase GABA-stimulated chloride influx, inverse agonists decrease GABA-stimulated chloride influx, and antagonists have no effect alone, but inhibit binding of

agonists and inverse agonists. Barbiturates also increase chloride influx through activated channels. Picrotoxin and other convulsants such as pentylenetetrazol and TBPS (tert-butyl bicyclo[2.2.2] phosphorothionate) block chloride flux presumably by directly blocking the ion channel.

Concentrations of PTZ required for binding at the picrotoxin site of the GABA receptor complex are similar to those observed *in vivo* during convulsions, suggesting that the convulsant effect of PTZ is due to this action (Ramanjaneyulu and Ticku, 1984). In addition, drugs which act on the GABA receptor complex to increase chloride influx inhibit PTZ-induced convulsions.

II.B.3. *Strychnine*

Strychnine is an alkyloid present in the seeds of *Stychnos nux-vomica*, a tree native to India. It is used as a poison for rats and other animal pests. In research, strychnine has been used to model generalized seizures (Fisher, 1989). Convulsions elicited by strychnine differ in character from those elicited by PTZ; however, they do progress from myoclonic jerk to tonic limb extension. Face and forelimb clonus is absent in strychnine-treated animals. Strychnine-induced convulsions are less sensitive than PTZ-induced convulsions to commonly used anticonvulsants.

Although it affects other portions of the central nervous system, the brainstem and spinal cord are considered important sites for the convulsant action of strychnine

(Franz, 1980). For example, animals whose spinal cords have been transected (separating the brain from the rest of the central nervous system) are still sensitive to strychnine-induced convulsions.

Strychnine increases the level of neural excitability by interfering with central inhibitory processes. Strychnine antagonizes glycine binding to its receptor (Curtis, Duggan and Johnston, 1971). Glycine is found in highest concentrations in the brainstem and spinal cord (McGeer and McGeer, 1989). It acts as an inhibitory neurotransmitter by increasing chloride ion conductance, thus causing postsynaptic hyperpolarization. It appears that the binding sites for glycine and strychnine are different; however, they interact allosterically (Marvizón, Vázquez, Calvo, Mayor, Gómez, Valdivieso and Benavides, 1986). Glycine has been shown to prevent strychnine-induced convulsions (Beyer, Banas, Gomora and Komisaruk, 1988; Halsey, Little and Wardley-Smith, 1989). In another study, however, glycine was shown to potentiate strychnine-induced convulsions (Larson and Beitz, 1988). This may have reflected an action of glycine upon the strychnine-insensitive glycine receptor which is allosterically linked to the excitatory amino acid *N*-methyl-D-aspartate (NMDA) receptor.

II.B.4. *Kainic acid*

Kainic acid was originally isolated from a Japanese seaweed for use as an ascarcide (anti-roundworm treatment); however, its present use is principally in neurobiological research. Kainic acid is used to model complex partial (limbic) seizures (reviewed by Patel, 1988). Administration to laboratory animals results in an initial period of arrested activity followed by complex motor activity, wild running clonus, and tonic limb extension. Kainic acid selectively destroys hippocampal neurons in a pattern that resembles the damage observed in human temporal lobe epilepsy.

EEG changes shortly following administration of kainic acid are restricted to limbic areas such as the hippocampus, amygdala or entorhinal cortex. Epileptiform activity spreads to non-limbic areas; however, it is clear that kainic acid-induced convulsions are initiated within limbic structures prior to propagation to cortical and other motor areas. Glucose utilization is increased in limbic structures following kainic acid administration (Lothman and Collins, 1981). Interestingly, diazepam, which attenuates kainic acid-induced convulsions, does not inhibit the increased glucose utilization in hippocampus (reviewed by Nadler, 1981). This suggests that diazepam suppresses the propagation, but not the initiation, of hippocampal seizures.

Kainic acid is an analogue of the excitatory amino acid neurotransmitter, glutamate. Glutamate increases neuronal permeability for Na^+ and other ions, and results in cell depolarization (reviewed by McGeer and McGeer, 1989). There are four main receptor types which bind excitatory amino acids: NMDA, quisqualate, kainate and L-aminophosphonobutyric acid receptors. The kainate receptor is activated by kainic acid and appears to be important for excitotoxicity. Action at this receptor excites neurons so powerfully that high levels of excitatory amino acids or kainic acid can result in neuronal destruction.

III. EXPERIMENTS

III.A. Rationale

There is a considerable literature suggesting that corticosteroids have both convulsant (or proconvulsant) and anticonvulsant actions. The review of the literature above revealed a trend toward excitatory effects of low doses of corticosterone and mineralocorticoids and inhibitory effects of high doses of corticosterone and glucocorticoids. Because of their binding characteristics, type I receptors would be expected to mediate effects of low corticosterone concentrations and mineralocorticoids, while type II receptors would be expected to mediate effects of high corticosterone concentrations and glucocorticoids. This has led to the hypothesis that type I receptors mediate

excitatory and type II receptors mediate inhibitory effects of corticosteroids on central nervous system excitability (Roberts, Chu, Crabbe and Keith, 1991; Joëls and de Kloet, 1992).

There is accumulating evidence from *in vitro* studies using type I and type II receptor antagonists which supports this hypothesis. For example, as was mentioned above, Joëls and de Kloet (1989) showed that high concentrations of corticosterone increased AHP amplitudes recorded from rat hippocampal slices. This inhibitory effect of corticosterone was reversed by the type II receptor antagonist, RU38486, and was mimicked by the type II agonist RU28362. In another study, this group showed that low concentrations of corticosterone decreased rat hippocampal AHP amplitudes (Joëls and de Kloet, 1990). This excitatory effect of corticosterone was reversed by the type I antagonist, spironolactone, and was mimicked by the type I agonist aldosterone.

Preliminary experiments from our laboratory support the hypothesis that type I receptors are involved in mediating the excitatory effect of corticosterone on convulsions elicited by PTZ (Roberts, Crabbe and Keith, in press (b)). Aminoglutethimide dose-dependently increased latencies to tonic hindlimb extension following 145 mg/kg PTZ administered intraperitoneally to ethanol Withdrawal Seizure Prone (WSP) mice. This anticonvulsant effect was reversed by a moderate dose of corticosterone and by the

mineralocorticoid deoxycorticosterone, but not by the glucocorticoid dexamethasone. In addition, the type I receptor antagonists spironolactone and RU26752 significantly increased latencies to PTZ-induced tonic hindlimb extension, suggesting that they have moderate anticonvulsant activity.

The purpose of these experiments was to examine the relative roles of type I and type II receptors in mediating corticosteroid effects on various convulsions in genetically heterogeneous mice. The convulsants pentylenetetrazol (PTZ), kainic acid, and strychnine were chosen based on their diverse mechanisms of action. At the present time, the studies which have best examined type I- versus type II-mediated corticosteroid effects were those performed using *in vitro* models. In addition, most of the reports which investigated corticosteroid effects *in vivo* or *in vitro* used a single measure of nervous system excitability. The present experiments may significantly contribute to this field as type I versus type II corticosteroid receptor effects was examined *in vivo* using several different types of convulsions.

Again, it was hypothesized that type I receptors mediate excitatory corticosteroid effects and type II receptors mediate inhibitory corticosteroid effects. The general approach used in the following experiments was as follows. For each convulsant, the effects of several doses of corticosterone were examined. Low doses were chosen to

attempt to maximize type I binding (present at circadian peak), while larger doses were included to maximize both type I and type II binding (present during a moderate state of stress). If corticosterone significantly altered doses of convulsant required for convulsions, the effects of the type I antagonist, spironolactone and the type II antagonist, RU38486 on this effect were then examined. In order to examine the effect of decreasing endogenous corticosteroid levels on convulsions, the steroid synthesis inhibitor, aminoglutethimide was employed. A dose-response study examining the effects of aminoglutethimide on convulsions was performed for each convulsant. If aminoglutethimide altered the dose of convulsant required for convulsions, it was determined whether replacement corticosterone was able to reverse this effect. Furthermore, the effect of the type I and type II antagonists on this reversal was also examined.

It was expected that a low dose of corticosterone would activate type I receptors, increasing excitability, and thereby decrease the doses of convulsant required for convulsions. A higher dose of corticosterone would activate type II receptors, decreasing excitability, and thereby increase the doses of convulsant required for convulsions. Type I antagonism was expected to block the excitatory effect of the low corticosterone dose. Type II antagonism was expected to block the inhibitory effect of the higher corticosterone dose. Aminoglutethimide would be expected to

decrease endogenous type I binding. Type II binding would not be expected to be affected as these receptors are not typically bound in the non-stressed state due to their lower affinity for corticosterone. Therefore, it was predicted that aminoglutethimide would increase doses of convulsants required for convulsions. The effect was expected to be reversed by replacement corticosterone treatment. The reversal was expected to be blocked by the type I receptor antagonist.

In addition to investigating relative type I and type II receptor actions, an examination of the effects of corticosteroid manipulations on each convulsant sign for each drug made it possible to determine whether corticosteroid effects on nervous system excitability are global or whether they display neuroanatomical and/or pharmacological specificity. Based on the wide variety of measures affected by corticosteroid manipulations (reviewed above), it was hypothesized that corticosteroid effects are global and that convulsions induced by each of the convulsants would be similarly affected by the corticosteroid manipulations. However, it is possible that corticosteroid effects are not global. For example, because type I and type II corticosteroid receptors are colocalized in the hippocampus, this may be a site of modulation of neural activity by corticosteroids. It may then be expected that kainic acid-induced convulsions, which are generally acknowledged to be limbic, would be sensitive to

corticosteroid manipulations. In addition, PTZ-induced clonus, which has been hypothesized to originate in the forebrain, may also be sensitive to corticosteroid manipulation. On the other hand, if corticosteroids interact either directly or indirectly with GABAergic neurotransmission, a different outcome would be predicted. PTZ-induced convulsions would be affected by corticosteroid manipulations in this case. Convulsions elicited by kainic acid and strychnine may also be affected, as GABA has been implicated in modulating general seizure propagation, perhaps through GABAergic outputs from the substantia nigra (Gale, 1988). In the Discussion section, possible explanations for the experimental results will be considered.

III.B. General methods

III.B.1. *Subjects*

Genetically heterogeneous mice were kindly supplied by Dr. E.J. Gallaher, Department of Veterans Affairs Medical Center, Portland, Oregon. These mice are maintained as controls for a breeding program in which mice are genetically selected for greater or lesser impairment on the rotarod apparatus following administration of diazepam. The foundation population consisted of HS (genetically heterogeneous) mice produced from matings between 8 inbred strains; A, AK, BALB/c, C3H, C57BL, DBA/2, Is/Bi, and RIII

(discussed in McClearn, Deitrich and Erwin, 1978). The protocol for matings of control mice is as follows.

For each generation, one male and one female mice was randomly selected from each of ten families without regard for benzodiazepine sensitivity. These mice were then bred with mice from another family, avoiding sibling and first-cousin matings, to establish ten new breeding pairs. Mice from the first litter were randomly selected to initiate the next generation. Untested mice from this litter as well as the next three litters produced by each breeding pair were used for various experiments. This breeding protocol was independently replicated from the same foundation stock. Mice used presently were from both replicate lines; generations 8 (4 families, litter number 4), 9 (10 families, litters number 1-4), and 10 (8 families, litter number 1).

Male mice between 60 and 120 days of age (25-35 g body weight) were used in these experiments. Mice were housed 2-4 per cage (12" x 6" polycarbonate boxes) containing corn cob bedding. Food and water were available ad libitum. Lights were on a 12/12 hour light/dark cycle, with lights on at 6:00 am. All experiments were initiated between 9:00 and 9:30 am. Mice used in the present experiments were naive to any treatment. Animal care and use was approved by the Animal Research Facility of the Department of Veterans Affairs Medical Center and was in compliance with NIH and USDA guidelines.

III.B.2. *Drug and hormone sources and preparation*

Corticosterone (Sigma Chemical Co.), aminoglutethimide (Sigma Chemical Co.), spironolactone (Sigma Chemical Co.), and RU38486 (Roussel-Uclaf, Romainville, France) were dissolved in dimethyl sulfoxide (DMSO-Mallinckrodt Specialty Chemicals Co.) and diluted in sesame oil. The final concentration of DMSO in sesame oil was 7%. Drug concentrations for each experiment varied between 0.05 to 2.5 mg/ml, depending on dose. These drugs were administered at a volume of 0.01 ml per gram body weight subcutaneously in the nape of the neck. This method of bolus administration extends the time in which drug levels are elevated relative to those observed following a single intravenous or intraperitoneal injection (unpublished observations). In cases in which mice received more than one of the above drugs, the injection sites were alternated between the left and right sides of the neck.

Pentylenetetrazol (Aldrich) and strychnine hemisulfate salt (Sigma Chemical Co.) were dissolved in 0.9% NaCl. Kainic acid (Sigma Chemical Co.) was dissolved in distilled H20. The concentrations of these drugs in solution were 5 mg/ml (PTZ and kainic acid) and 0.05 mg/ml (strychnine). The infusion rate for these three convulsants was 0.5 ml/min.

III.B.3. *Timed intravenous convulsant infusions*

Animals were removed from their home room to a procedure room and weighed and marked before initiation of the experiment. One mouse was injected subcutaneously with hormone every three minutes for 1 hour. Twenty mice were tested each day. In some cases a second or third pre-injection occurred during hour 2. One hour after the final pre-injection, each mouse was taken from its home cage, placed in a clear Plexiglas cylinder, and restrained with its tail free. The tail of the mouse was inserted into 47°C water for 1 minute in order to dilate the veins. The mouse restrainer was then firmly attached to a ring stand. A 27x3/8 butterfly infusion needle was inserted in one of the two lateral tail veins of the mouse. A foot switch was depressed to start the infusion pump (Sage Instruments) and a digital timer (GraLab). Correct placement of the needle was verified by the appearance of blood in the infusion tubing which disappeared upon infusion, or by whitening of the vein during infusion. The mouse was observed during infusion and latencies (seconds) to each convulsion sign were recorded vocally into a tape recorder. Following the endpoint convulsion, the foot switch was depressed to stop the timer and pump. Mice were immediately cervically dislocated and decapitated for collection of blood for corticosterone determinations.

III.B.4. *Scoring of convulsions*

Five types of convulsion were observed during administration of the convulsant drugs. These were either tonic or clonic in nature. Tonic or tonus refers to rigidity due to muscle contraction. Clonic or clonus refers to rhythmic contraction and relaxation of muscles. The five types of convulsions were: (1) Myoclonic jerk, characterized by a sudden involuntary muscle twitch often accompanied by head twitch and/or squeak; (2) Face and forelimb clonus, characterized by rapid writhing movements of the head and neck, and clonic forelimb movements; (3) Running bouncing clonus, characterized by a violent whole body clonus, usually terminating in tonic hindlimb extension; (4) Wild running clonus, characterized by running movements of the limbs, generally starting with one forelimb, extending to the other, and finally involving all limbs, and; (5) Tonic hindlimb extension (THE), characterized by extreme rigidity, caudal extension of forelimbs and hindlimbs, head held perpendicular to the body, and ears laid back and eyes closed. THE was often terminal.

The progression of seizures produced by each convulsant was unique. PTZ produced myoclonus, followed rapidly by face and forelimb clonus. The mouse was then quiet until it displayed running bouncing clonus and tonic hindlimb extension. Strychnine produced myoclonus followed by a very rapid bout of running bouncing clonus. Death often occurred simultaneous to THE. Kainic acid caused wild running clonus

followed rapidly by THE. Latencies to each of these convulsions were recorded.

III.B.5. *Corticosterone radioimmunoassay*

Trunk blood (approximately 20 µl) was collected in heparinized capillary tubes. The samples were centrifuged at 2000 rpm for 20 min. Five µl plasma was removed and diluted with 100 µl sterile water and then stored at 4°C until assayed. Radioimmunoassay was adapted from that previously described by Keith, Winslow, and Reynolds (1978) and is described briefly below.

The experimental samples were immersed in boiling water to denature corticosterone binding globulin (Murphy, 1963), which would compete with the antibody for binding with corticosterone. Duplicate standard solutions were made containing 0, 10, 20, 50, 100, 200, 500, 1,000, 2,000, and 10,000 pg corticosterone in 100 µl buffer. Tubes were also prepared to estimate the total binding capacity and non-specific binding of the assay. One hundred µl (equal to 10,000 counts per min) [^{125}I]-corticosterone (ICN) and 100 µl corticosterone antibody (Ventrex), titrated to bind approximately 40% of the total [^{125}I]-corticosterone, were added to the samples and standards. After vortexing, the tubes were incubated at 4°C for approximately 24 hr. Five hundred µl dextran-coated charcoal (4°C) was added to each tube (except the total count tubes). Following a 10 min incubation period, the tubes were centrifuged in order to

separate free from antibody-bound corticosterone. The supernatant was decanted into new test tubes and counted (Micromedic Automatic Gamma Counter).

Counts per minute were normalized and fit to a least-squares fit regression equation produced by log-logit transformation of the standards. The smallest concentration of corticosterone producing a response different from the zero response at the 95% confidence interval was 0.1 µg/dl. The maximal detectable corticosterone concentration was 400 µg/dl. Intra-assay variability was less than 10%. The specificity of this assay was very high with only 4% cross-reactivity to desoxycorticosterone, 1% cross-reactivity to 5 β-pregnanedione, and less than 0.6% cross-reactivity to other adrenal steroids.

III.B.6. *Data Analysis*

Data were analyzed using analysis of variance (ANOVA). Data from each convulsion sign (doses of convulsant required for convulsion and plasma corticosterone levels) were analyzed separately. When main effects were significant, post-hoc comparisons were made using the Newman-Keuls statistic. Simple main effects analyses were performed on significant two-way interactions (Keppel, 1991). These were followed by additional simple comparisons if more than two groups were involved. Comparisons were considered significant at $p < 0.05$.

III.C. Experimental methods

III.C.1. *Pentylenetetrazol*

Experiment 1:

The purpose of Experiment 1 was to examine the effects of various doses of corticosterone on PTZ-induced convulsions. Thirty two mice received either vehicle, 0.5, 1, or 5 mg/kg corticosterone (n = 8/group). One hour later, latencies to convulsions following PTZ infusion were recorded. Immediately following THE, mice were decapitated and trunk blood was collected for corticosterone determinations.

Experiment 2:

The results of Experiment 1 suggested that 0.5 mg/kg corticosterone significantly decreased the dose of PTZ required for myoclonic jerk. The purpose of this experiment was to examine the ability of type I and type II corticosteroid receptor antagonists to block this effect of corticosterone. If type I receptors are important for excitatory effects of corticosteroids, it would be expected that spironolactone, the type I antagonist, would block the convulsant effect of 0.5 mg/kg corticosterone. In addition, the effects of the antagonists on PTZ-induced convulsions in vehicle-treated mice were examined.

Thirty nine mice were used in this experiment. Twelve to fifteen mice were injected with vehicle, 5 mg/kg spironolactone, or 5 mg/kg RU38486. This was immediately

followed by injection of either 0 or 0.5 mg/kg corticosterone (n = 6-8/group). One hour later PTZ was infused and latencies to convulsions were recorded. Trunk blood was collected for corticosterone determinations.

Experiment 3:

The purpose of Experiment 3 was to examine the effects of various doses of aminoglutethimide, a steroid synthesis inhibitor, on PTZ-induced convulsions. Twenty four mice were injected with either 0, 6.25, 12, 25, or 50 mg/kg aminoglutethimide (n = 4-6/group). One hour later, latencies to PTZ-induced convulsions were recorded. Blood was sampled for corticosterone determinations.

Experiment 4:

The results of Experiment 3 suggested that 25 and 50 mg/kg aminoglutethimide increased the doses of PTZ required for myoclonic jerk and face and forelimb clonus. Aminoglutethimide did not significantly affect running bouncing clonus or tonic hindlimb extension. The purpose of Experiment 4 was to determine whether 1 mg/kg corticosterone could reverse the anticonvulsant effect of 25 mg/kg aminoglutethimide. Although 0.5 mg/kg corticosterone was effective in Experiment 1, aminoglutethimide-treated mice were expected to have lower endogenous corticosterone levels than non-aminoglutethimide-treated mice, therefore a higher dose was chosen.

Thirty nine mice were injected with either 0 or 25 mg/kg aminoglutethimide. One hour later, vehicle or 1 mg/kg corticosterone was administered to half of the animals from each group (n = 9-10/group). Following another hour, PTZ was infused and convulsion latencies were recorded. Blood was sampled for corticosterone determinations.

Experiment 5:

The results of Experiment 4 suggested that 1 mg/kg corticosterone was able to reverse the anticonvulsant effect of 25 mg/kg aminoglutethimide. The purpose of Experiment 5 was to examine the effects of the type I and type II antagonists on this reversing effect of corticosterone. Type I receptors are hypothesized to be important in mediating excitatory effects of corticosterone, therefore it was expected that the type I antagonist would block the reversing effect of corticosterone in this experiment.

Fifty nine mice were injected with 25 mg/kg aminoglutethimide. One hour later, these mice received either vehicle (n = 22), 5 mg/kg spironolactone (n = 18), or 5 mg/kg RU38486 (n = 19). These injections were immediately followed by either 0 or 1 mg/kg corticosterone (n = 9-11/group). After another hour, PTZ was infused and convulsion latencies were recorded. Blood was sampled for corticosterone determinations.

III.C.2. *Strychnine*

Experiment 6:

The purpose of this experiment was to examine the effects of corticosterone on strychnine-induced convulsions. Forty nine mice received either 0 (n = 15), 0.5 (n = 14), 1 (n = 9), or 5 (n = 11) mg/kg corticosterone. One hour later, strychnine was infused and latencies to convulsions were recorded. Trunk blood was collected for corticosterone RIA.

Experiment 7:

The results of Experiment 6 suggested that none of the doses of corticosterone tested significantly altered doses of strychnine required for convulsions. Therefore, it was not of interest to examine the effects of the corticosteroid receptor antagonists in combination with corticosterone. The purpose of Experiment 7 was to investigate the effects of the type I and type II corticosteroid receptor antagonists administered alone on strychnine-induced convulsions. It was hypothesized that spironolactone and RU38486 would have no effect, as the results of the previous experiment suggested that these convulsions are corticosterone-insensitive.

Nineteen mice were injected with either vehicle (n = 7), 5 mg/kg spironolactone (n = 6), or 5 mg/kg RU38486 (n = 6). One hour later, strychnine was infused and latencies to convulsions were recorded. Trunk blood was collected for

corticosterone determinations following tonic hindlimb extension.

Experiment 8:

The purpose of Experiment 8 was to examine the effects of various doses of aminoglutethimide on strychnine-induced convulsions. Twenty three mice received either 0, 6.25, 12.5, 25, or 50 mg/kg aminoglutethimide (n = 3-5/group). One hour later, strychnine was infused and latencies to convulsions were recorded. Blood was collected for corticosterone determinations.

III.C.3. *Kainic acid*

Experiment 9:

The purpose of this experiment was to examine the effects of corticosterone on kainic acid-induced convulsions. Nineteen mice were injected with either vehicle, 0.5, 1, or 5 mg/kg corticosterone (n = 4-5/group). One hour later, kainic acid was infused and latencies to convulsions were recorded. Trunk blood was collected for corticosterone RIA.

Experiment 10:

The results of Experiment 9 suggested that 1 and 5 mg/kg corticosterone significantly reduced the dose of kainic acid required for convulsions. The purpose of this experiment was to examine the ability of the type I and type

II antagonists to block the effect of 1 mg/kg corticosterone on kainic acid convulsions. If type I receptors are important for excitatory effects of corticosteroids, it would be expected that spironolactone, the type I antagonist, would block the convulsant effect of corticosterone. In addition, the effects of the antagonists on kainic acid-induced convulsions in vehicle-treated mice was examined.

Thirty five mice were used in this experiment. Mice were injected with vehicle (n = 14), 5 mg/kg spironolactone (n = 11), or 5 mg/kg RU38486 (n = 10). This was immediately followed by injection of either 0 or 1 mg/kg corticosterone (n = 5-7/group). One hour later kainic acid was infused and latencies to convulsions were recorded. Trunk blood was collected for corticosterone determinations.

Experiment 11:

The purpose of Experiment 11 was to examine the effects of aminoglutethimide on kainic acid-induced convulsions. Twenty three mice received either 0, 6.25, 12.5, 25, or 50 mg/kg aminoglutethimide (n = 4-5/group). One hour later, kainic acid was infused and latencies to convulsions were recorded. Blood was collected for corticosterone determinations.

Experiment 12:

The results of Experiment 11 suggested that 50 mg/kg aminoglutethimide increased the doses of kainic acid required for wild running clonus and tonic hindlimb extension. Therefore, the purpose of this experiment was to examine whether 2 mg/kg corticosterone could reverse the anticonvulsant effect of aminoglutethimide and whether the type I and type II antagonists blocked this reversal. Although 1 mg/kg corticosterone was effective in Experiment 8, aminoglutethimide-treated mice were expected to have lower endogenous corticosterone levels than non-aminoglutethimide-treated mice, therefore a higher dose was chosen. Type I receptors are hypothesized to be important in mediating excitatory effects of corticosterone, therefore it was expected that the type I antagonist would block the reversing effect of corticosterone in this experiment.

Thirty six mice received 50 mg/kg aminoglutethimide. One hour later, twelve mice were injected with either vehicle, 5 mg/kg spironolactone, or 5 mg/kg RU38486, followed immediately by either 0 or 2 mg/kg corticosterone (n = 6/group). Following another hour, the mice were infused with kainic acid and latencies to convulsions were recorded. Blood was sampled for corticosterone determinations.

IV. RESULTS

IV.A. Pentylenetetrazol

Experiment 1:

In Experiment 1, the effect of corticosterone on pentylenetetrazol (PTZ)-induced convulsions was examined. The results of this experiment are shown in Table 3. These results suggested that PTZ-induced myoclonic jerk was sensitive to the proconvulsant effect of corticosterone. Face and forelimb clonus, running bouncing clonus, and tonic hindlimb extension were insensitive to modulation by corticosterone at the doses tested presently.

There was a significant effect of corticosterone on dose of PTZ required for myoclonic jerk ($F(3,28) = 6.67$, $p < 0.01$). Post-hoc analysis revealed that 0.5 mg/kg corticosterone decreased the dose of PTZ required for this convulsion sign ($p < 0.01$). No other convulsion sign was significantly affected by corticosterone at the doses tested (face and forelimb clonus, $F(3,28) = 0.96$, $p = 0.43$; running bouncing clonus, $F(3,28) = 2.08$, $p = 0.12$; tonic hindlimb extension, $F(3,28) = 0.96$, $p = 0.43$).

Plasma corticosterone levels measured following tonic hindlimb extension are also shown in Table 3. There was a significant effect of corticosterone on plasma hormone levels ($F(3,28) = 108.69$, $p < 0.0001$). Each dose significantly increased plasma corticosterone levels over each lower dose ($p < 0.05$).

Table 3. Doses of PTZ (mg/kg) required for convulsions and plasma corticosterone levels (µg/dl) in mice following corticosterone preinjection.

Corticosterone (mg/kg)	Myoclonic jerk	Face & forelimb clonus	Running bouncing clonus	Tonic hindlimb extension	Plasma cortico-sterone (µg/dl)
0	34.0 ± 1.7	41.0 ± 2.8	91.6 ± 4.3	105.7 ± 7.7	4.0 ± 0.6
0.5	26.5 ± 1.8*	36.5 ± 4.1	80.4 ± 10.2	94.2 ± 12.0	12.7 ± 0.7*
1	35.7 ± 1.4	43.7 ± 2.3	95.5 ± 11.2	104.8 ± 11.9	16.7 ± 1.2*
5	34.2 ± 1.4	43.8 ± 4.4	69.9 ± 3.0	85.3 ± 6.7	35.2 ± 2.0*

PTZ doses and plasma corticosterone levels are expressed as means ± SEM. There was a significant effect of corticosterone dose on dose of PTZ required for myoclonic jerk and plasma corticosterone levels ($p < 0.01$). * Significantly different from 0 mg/kg corticosterone ($p < 0.05$).

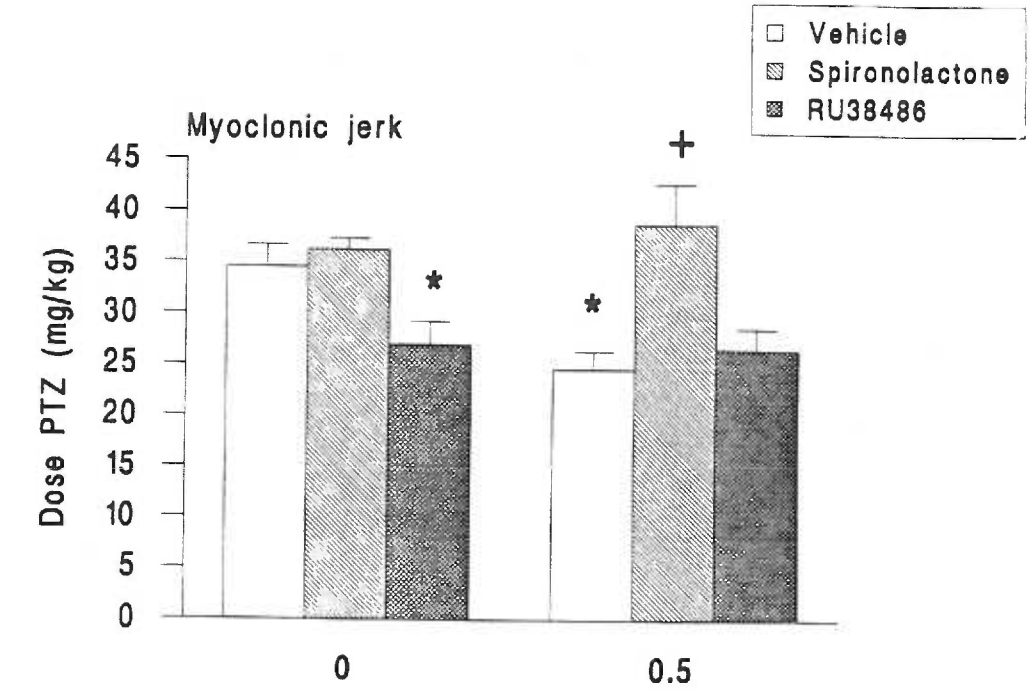
Experiment 2:

The purpose of Experiment 2 was to determine whether the proconvulsant effect of 0.5 mg/kg corticosterone upon PTZ-induced myoclonic jerk was reversed by either spironolactone (type I corticosteroid receptor antagonist) or RU38486 (type II receptor antagonist). The results of this experiment are shown in Figures 2 and 3. These results suggested that spironolactone reversed the proconvulsant effect of 0.5 mg/kg corticosterone on PTZ-induced myoclonic jerk. Corticosterone significantly decreased the dose of PTZ required for myoclonic jerk in vehicle-treated mice. This effect was blocked in spironolactone-treated mice. RU38486 appeared to have a proconvulsant effect.

Overall, doses of PTZ required for convulsions were higher in spironolactone-treated mice than those treated with vehicle or RU38486 ($p < 0.01$). There was a statistically significant main effect of antagonist treatment on doses of PTZ required for myoclonic jerk ($F(2,33) = 10.74$, $p < 0.001$), face and forelimb clonus ($F(2,33) = 9.98$, $p < 0.001$), running bouncing clonus ($F(2,33) = 8.76$, $p < 0.001$), and tonic hindlimb extension ($F(2,33) = 9.99$, $p < 0.001$).

Although close in some cases, corticosterone did not significantly affect doses of PTZ required for myoclonic jerk ($F(1,33)= 2.9$, $p = 0.09$), face and forelimb clonus ($F(1,33) = 1.14$, 0.29), running bouncing clonus ($F(1,33) =$

Figure 2. Effect of corticosteroid receptor antagonists on PTZ-induced myoclonic jerk and face and forelimb clonus in mice treated with 0 or 0.5 mg/kg corticosterone. Bars represent means ± SEM. (* significantly different from 0 corticosterone - Vehicle group. + significantly different from 0.5 mg/kg corticosterone - Vehicle group).


Vehicle
Spironolactone
RU38486
Myoclonic jerk
Dose PTZ (mg/kg)
45
40
35
30
25
20
15
10
5
0
*
*
+
0
0.5

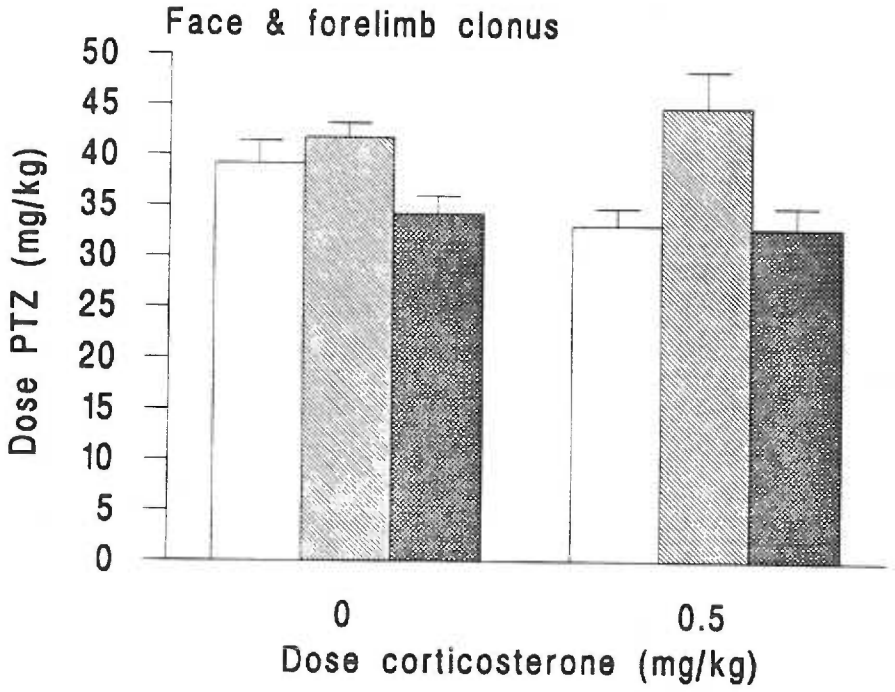
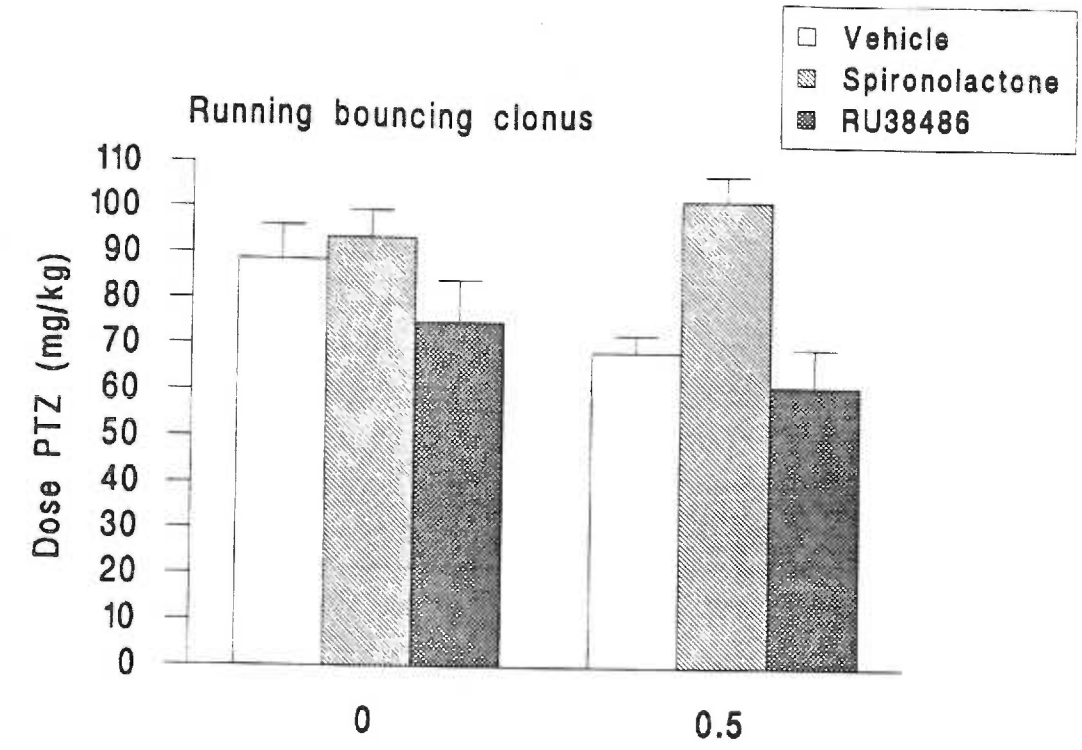
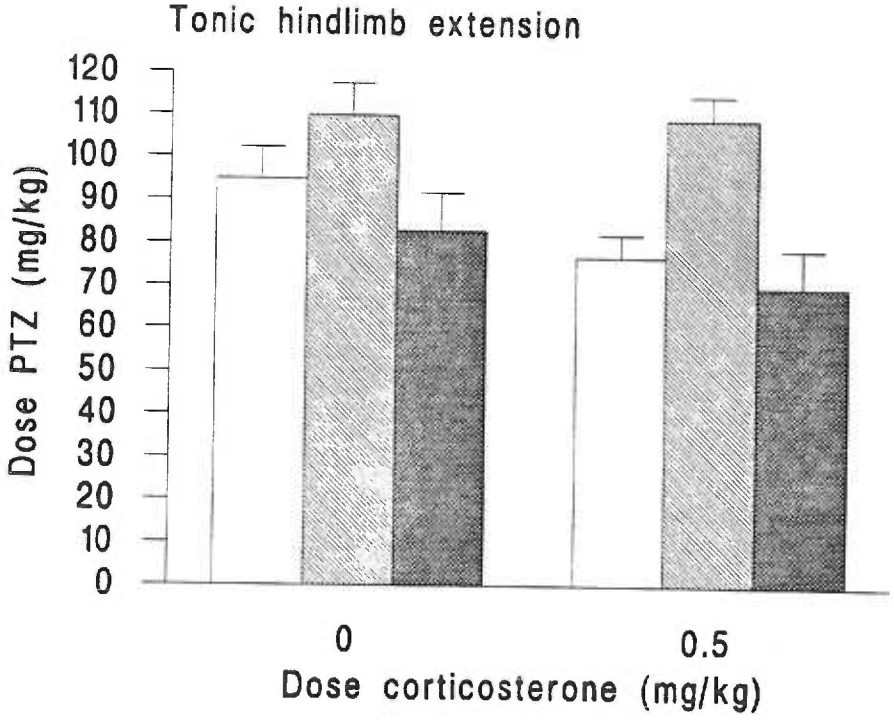

Face & forelimb clonus
Dose PTZ (mg/kg)
50
45
40
35
30
25
20
15
10
5
0
0
0.5
Dose corticosterone (mg/kg)

Figure 3. Effect of corticosteroid receptor antagonists on PTZ-induced running bouncing clonus and tonic hindlimb extension in mice treated with 0 or 0.5 mg/kg corticosterone. Bars represent means ± SEM.


Vehicle
Spironolactone
RU38486
Running bouncing clonus
Dose PTZ (mg/kg)
110
100
90
80
70
60
50
40
30
20
10
0
0
0.5


Tonic hindlimb extension
Dose PTZ (mg/kg)
120
110
100
90
80
70
60
50
40
30
20
10
0
0
0.5
Dose corticosterone (mg/kg)

2.75, $p = 0.11$), and tonic hindlimb extension ($F(1,33) = 3.55$, $p = 0.07$).

There was a significant corticosterone by antagonist interaction for PTZ-induced myoclonic jerk ($F(2,33) = 4.13$, $p < 0.05$). Simple main effects analysis revealed that corticosterone significantly decreased PTZ doses required for myoclonic jerk in the vehicle-treated group ($F(1,33) = 10.49$, $p < 0.001$). This effect of corticosterone was blocked by spironolactone treatment. In addition, simple main effects analyses also revealed significant effects of antagonist treatment in mice treated with 0 mg/kg corticosterone ($F(2,33) = 4.22$, $p < 0.025$) and 0.5 mg/kg ($F(2,33) = 11.17$, $p < 0.001$). Follow-up simple comparisons indicated that these effects were due to a decreased dose of PTZ required in mice treated with RU38486 in the 0 mg/kg corticosterone group ($p < 0.05$) and an increased dose of PTZ required in mice treated with spironolactone in the 0.5 mg/kg corticosterone group ($p < 0.001$). Antagonist by corticosterone interactions were not statistically significant for face and forelimb clonus ($F(2,33) = 2.14$, $p = 0.13$), running bouncing clonus ($F(2,33) = 2.29$, $p = 0.12$), and tonic hindlimb extension ($F(2,33) = 0.73$, $p = 0.49$).

Plasma corticosterone levels achieved in this experiment are shown in Table 4. There was a significant main effect of corticosterone on plasma hormone levels ($F(1,33) = 34.54$, $p < 0.0001$). Overall, 0.5 mg/kg

Table 4. Effects of corticosteroid antagonists ± corticosterone on plasma corticosterone levels (µg/dl) following PTZ-induced convulsions in mice.

Corticosterone (mg/kg)	Vehicle	Spironolactone	RU38486
0	10.9 ± 1.8	6.9 ± 2.5	7.6 ± 1.9
0.5	16.9 ± 2.3	19.8 ± 2.1	17.8 ± 1.2

Corticosterone levels are expressed as means ± SEM. There was a significant overall effect of corticosterone on plasma levels ($p < 0.0001$), but no effect of treatment with antagonist.

corticosterone significantly increased plasma corticosterone levels. There was no effect of antagonist treatment on this measure.

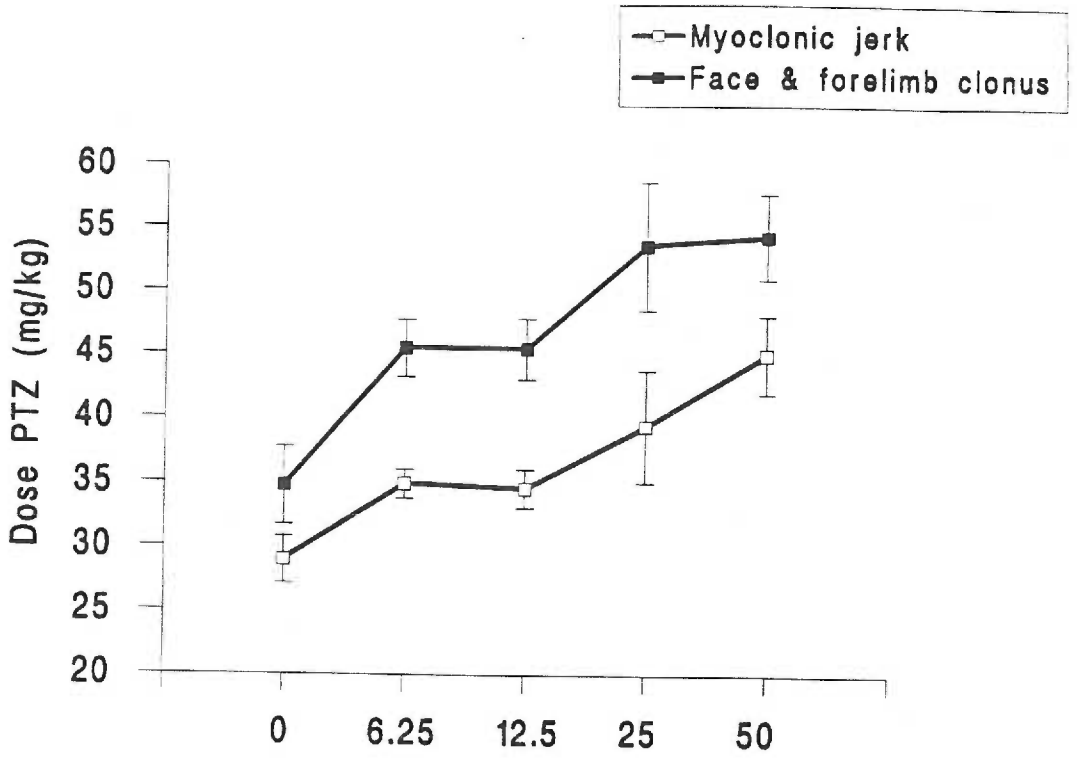
Experiment 3:

The purpose of Experiment 3 was to examine the effects of lowering corticosterone levels (with aminoglutethimide) on PTZ-induced convulsions. The results of this experiment are shown in Figure 4. These results suggested that the steroid synthesis inhibitor aminoglutethimide significantly increased doses of PTZ required for myoclonic jerk and face and forelimb clonus, while not affecting running bouncing clonus and tonic hindlimb extension. These effects occurred at aminoglutethimide doses which significantly decreased plasma corticosterone levels.

There were significant effects of aminoglutethimide on dose of PTZ required for myoclonic jerk ($F(4,19) = 5.86$, $p < 0.01$) and face and forelimb clonus ($F(4,19) = 6.37$, $p < 0.01$). The two highest doses of aminoglutethimide (25 and 50 mg/kg) significantly increased doses of PTZ required for these convulsion signs ($p < 0.05$). Aminoglutethimide did not significantly affect running bouncing clonus ($F(4,19) = 0.70$, $p = 0.60$) or tonic hindlimb extension ($F(4,19) = 1.27$, $p = 0.31$).

Plasma corticosterone levels obtained following tonic hindlimb extension are shown in Figure 5. There was a significant effect of aminoglutethimide on plasma

Figure 4. Dose-response effects of aminoglutethimide on PTZ-induced convulsions. Symbols represent means ± SEM.


Myoclonic jerk
Face & forelimb clonus
Dose PTZ (mg/kg)
60
55
50
45
40
35
30
25
20
0
6.25
12.5
25
50

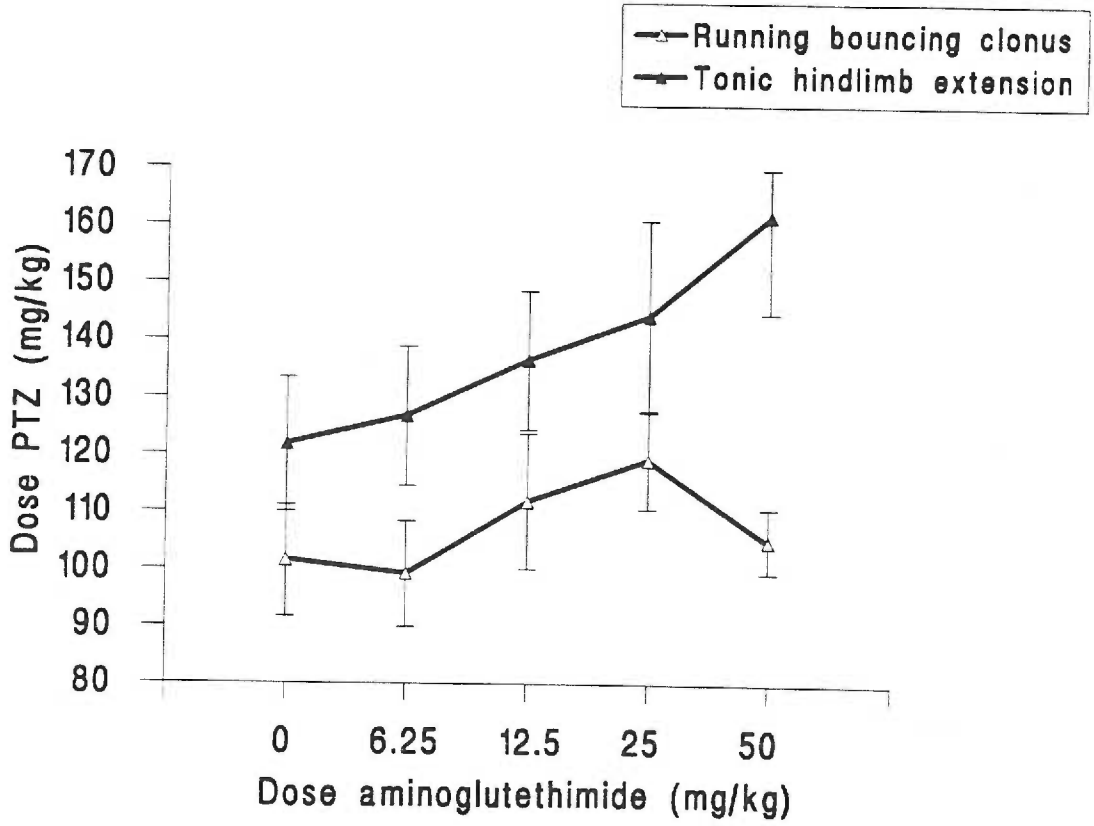
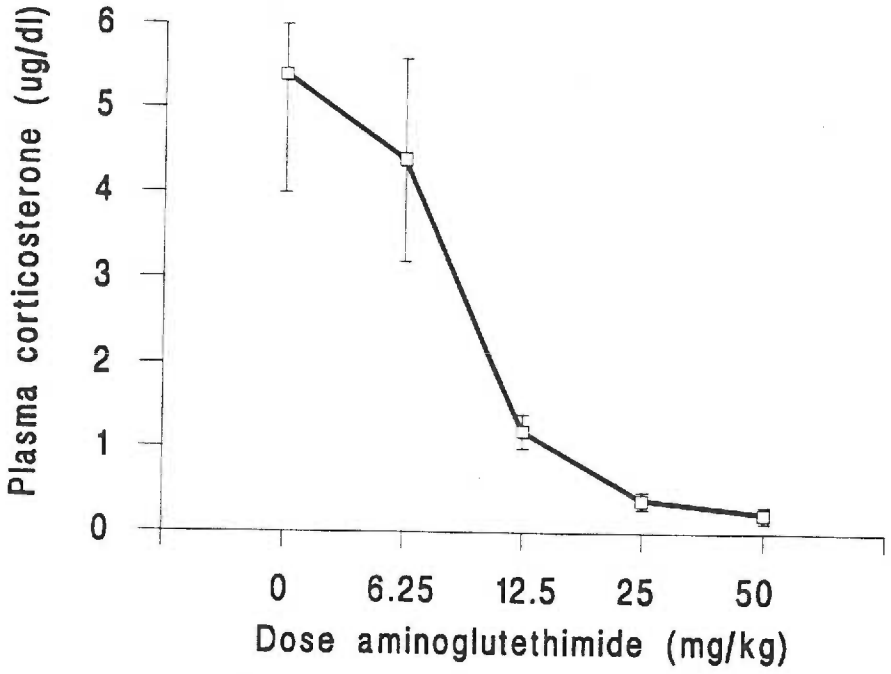

Running bouncing clonus
Tonic hindlimb extension
Dose PTZ (mg/kg)
170
160
150
140
130
120
110
100
90
80
0
6.25
12.5
25
50
Dose aminoglutethimide (mg/kg)

Figure 5. Dose-response effects of aminoglutethimide on plasma corticosterone levels. Blood was collected following PTZ-induced convulsions. Symbols represent means ± SEM.


Plasma corticosterone (ug/dl)
6
5
4
3
2
1
0
0
6.25
12.5
25
50
Dose aminoglutethimide (mg/kg)

corticosterone levels ($F(4,19) = 7.14$, $p < 0.01$). Corticosterone levels were decreased by 12.5 ($p < 0.05$), 25 ($p < 0.01$), and 50 ($p < 0.01$) mg/kg aminoglutethimide.

Experiment 4:

The purpose of Experiment 4 was to determine whether the anticonvulsant effect of 25 mg/kg aminoglutethimide was reversed by replacement corticosterone treatment. The results of this experiment are shown in Figures 6 and 7. These results suggested that 1 mg/kg corticosterone reversed the anticonvulsant effect of 25 mg/kg aminoglutethimide on PTZ-induced tonic hindlimb extension. This result is a bit confusing as this dose of aminoglutethimide did not affect tonic hindlimb extension in Experiment 3. This issue will be mentioned further in the Discussion.

There was a significant main effect of aminoglutethimide on PTZ doses required for myoclonic jerk ($F(1,35) = 19.92$, $p < 0.001$), face and forelimb clonus ($F(1,35) = 13.64$, $p < 0.001$), and tonic hindlimb extension ($1,35) = 8.59$, $p < 0.01$). Overall, 25 mg/kg aminoglutethimide increased doses of PTZ required for these convulsion signs. Again, running bouncing clonus was not significantly affected by aminoglutethimide ($F(1,35) = 1.70$, $p = 0.20$).

There were significant main effects of corticosterone treatment on doses of PTZ necessary to achieve myoclonic jerk ($F(1,35) = 9.52$, $p < 0.01$) and tonic hindlimb

Figure 6. Effects of 0 or 1 mg/kg corticosterone on PTZ-induced myoclonic jerk and face and forelimb clonus in mice pretreated with 0 or 25 mg/kg aminoglutethimide. Bars represent means ± SEM.

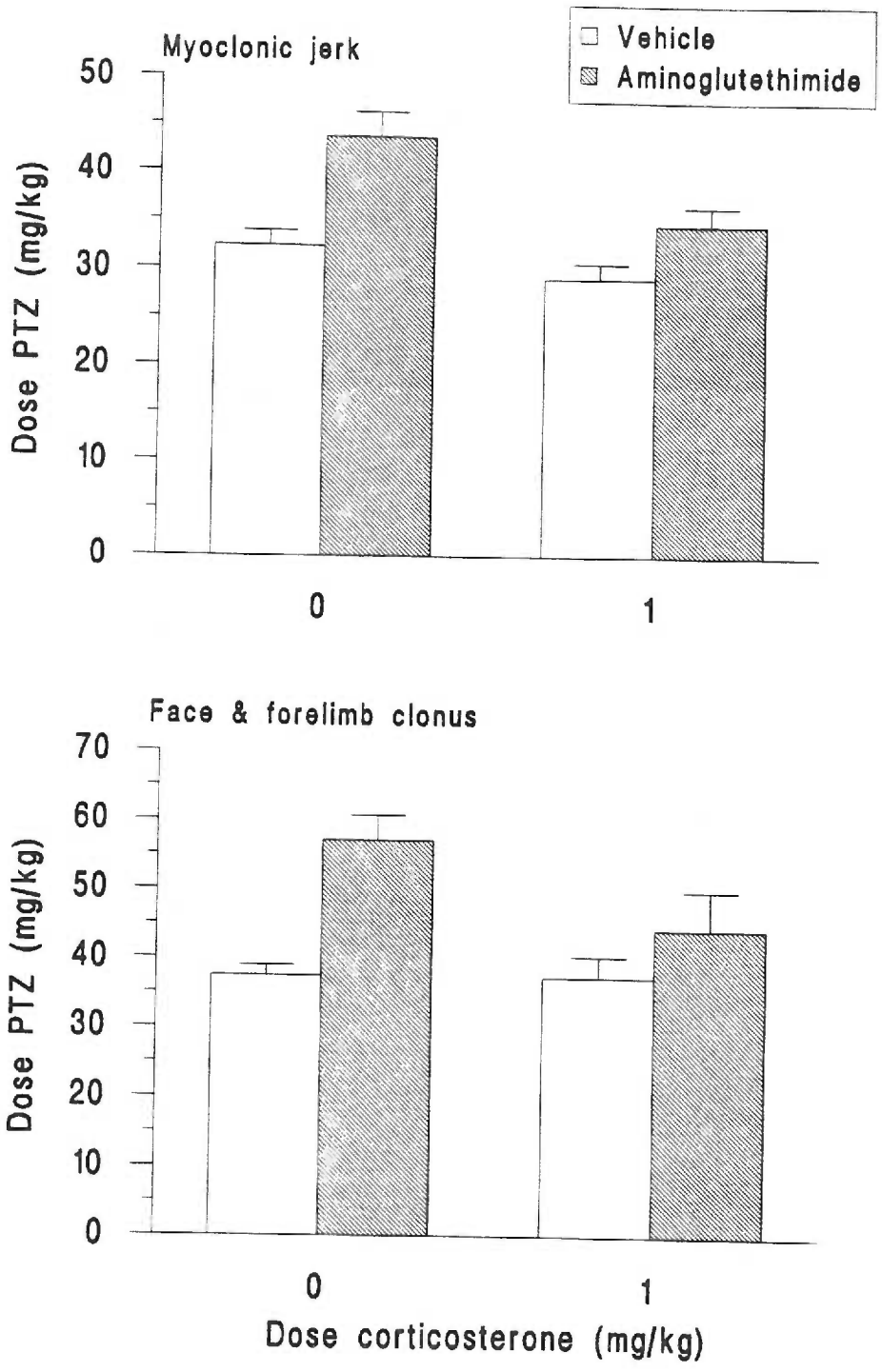
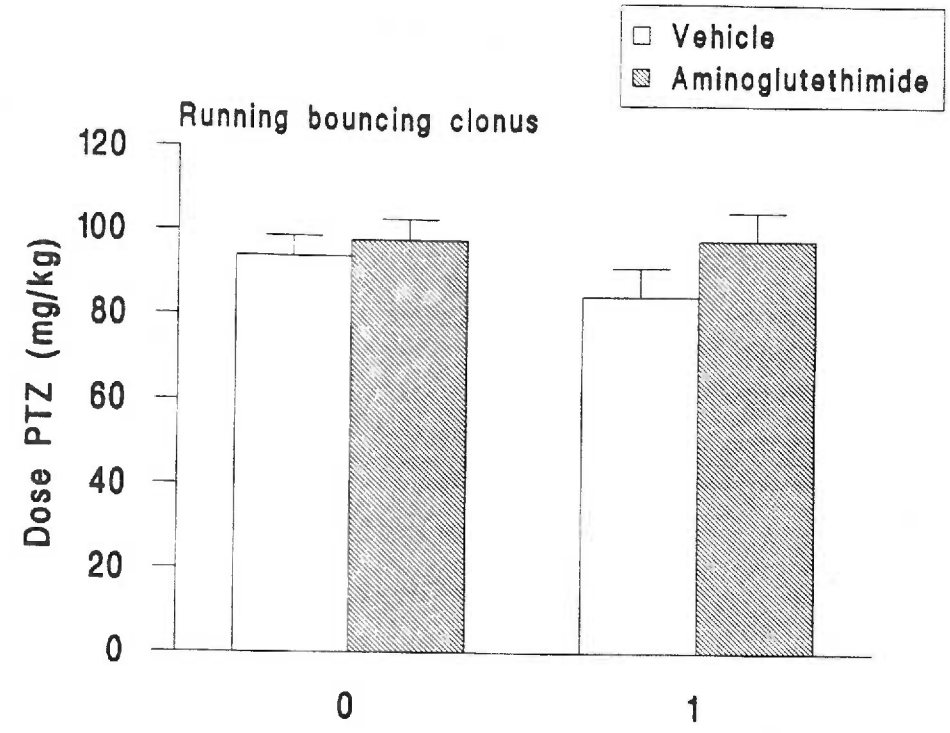
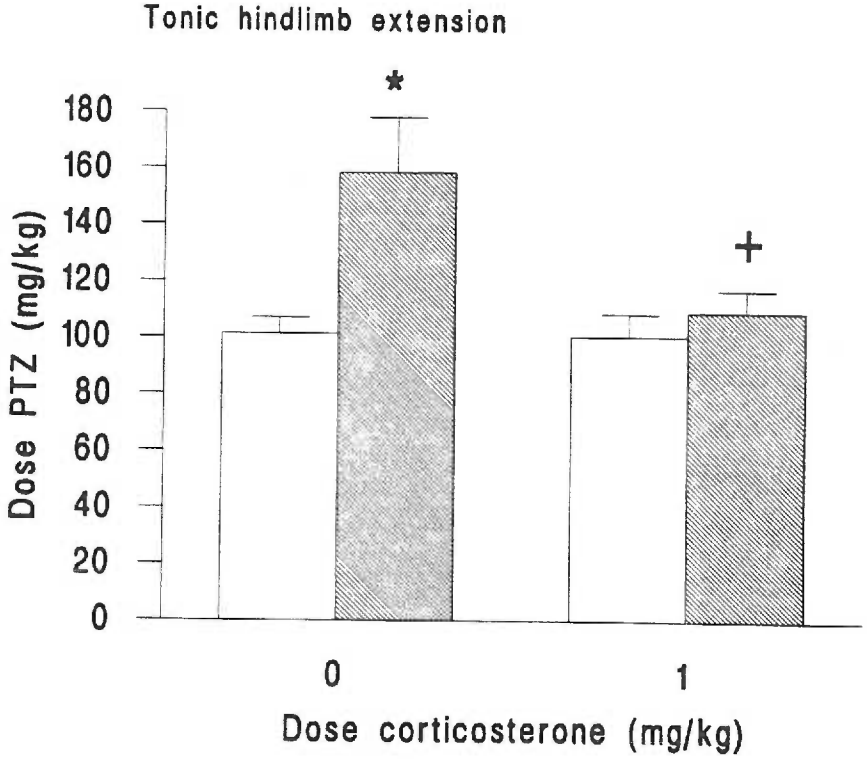

Myoclonic jerk
Vehicle
Aminoglutethimide
Dose PTZ (mg/kg)
50
40
30
20
10
0
0
1
Face & forelimb clonus
Dose PTZ (mg/kg)
70
60
50
40
30
20
10
0
0
1
Dose corticosterone (mg/kg)

Figure 7. Effects of 0 or 1 mg/kg corticosterone on PTZ-induced running bouncing clonus and tonic hindlimb extension in mice pretreated with 0 or 25 mg/kg aminoglutethimide. Bars represent means ± SEM. (* significantly different from 0 corticosterone - Vehicle group, + significantly different from 0 corticosterone - aminoglutethimide group).


Vehicle
Aminoglutethimide
Running bouncing clonus
120
100
80
60
40
20
0
Dose PTZ (mg/kg)
0
1


Tonic hindlimb extension
*
+
180
160
140
120
100
80
60
40
20
0
Dose PTZ (mg/kg)
0
1
Dose corticosterone (mg/kg)

extension ($F(1,35) = 4.35$, $p < 0.05$). Overall, 1 mg/kg corticosterone decreased doses of PTZ required for these convulsion signs. Corticosterone did not significantly affect face and forelimb clonus ($F(1,35) = 2.87$, $p = 0.09$) or running bouncing clonus ($F(1,35) = 0.46$, $p = 0.50$).

There was a significant aminoglutethimide by corticosterone interaction upon doses of PTZ required for tonic hindlimb extension ($F(1,35) = 4.32$, $p < 0.05$). The results of simple main effects analyses indicated that the aminoglutethimide + 0 mg/kg corticosterone group required higher PTZ doses than the group treated with vehicle + 0 mg/kg corticosterone ($F(1,35) = 11.71$, $p < 0.01$) or the group treated with aminoglutethimide + 1 mg/kg corticosterone ($F(1,35) = 8.69$, $p < 0.01$).

The interactions between aminoglutethimide and corticosterone were not significant in the analyses of PTZ doses required for myoclonic jerk ($F(1,35) = 2.06$, $p = 0.1$) and face and forelimb clonus ($F(1,35) = 2.96$, $p = 0.09$), although inspection of the figures is suggestive of a potential reversing effect of corticosterone on the anticonvulsant effect of aminoglutethimide.

Plasma corticosterone levels obtained following tonic hindlimb extension are shown in Table 5. There was a significant main effect of corticosterone on plasma corticosterone levels ($F(1,35) = 130.64$, $p < 0.0001$). There was no effect of aminoglutethimide ($F(1,35) = 1.65$, $p = 0.21$) or significant interaction between corticosterone and

Table 5. Plasma corticosterone concentrations (µg/dl) following PTZ-induced convulsions in mice pretreated with vehicle or 25 mg/kg aminoglutethimide and 0 or 1 mg/kg corticosterone.

Corticosterone (mg/kg)	Vehicle	Aminoglutethimide
0	5.1 ± 1.1	1.8 ± 1.1*
1	16.9 ± 0.9	17.9 ± 1.7

Plasma corticosterone concentrations are expressed as means ± SEM. * Significantly different from Vehicle group ($p < 0.05$, Student's t-test)

aminoglutethimide ($F(1,35) = 3.09$, $p = 0.09$), although corticosterone levels in aminoglutethimide-treated mice that did not receive corticosterone were lower than those of vehicle-treated mice. The large effect of corticosterone apparently obscured the aminoglutethimide effect, as a simple comparison between the two groups not given corticosterone revealed a significant effect of aminoglutethimide ($p < 0.05$, Student's t-test).

Experiment 5:

The purpose of Experiment 5 was to examine the effects of spironolactone (type I antagonist) and RU38486 (type II antagonist) on the reversing effect of 1 mg/kg corticosterone on PTZ-induced convulsions in aminoglutethimide-pretreated mice. The results of this experiment are shown in Figures 8 and 9. These results suggested that RU38486 had proconvulsant action whether corticosterone was present or not (except for tonic hindlimb extension). More importantly, these results suggested that spironolactone was able to inhibit the reversing effect of corticosterone on PTZ-induced myoclonic jerk, face and forelimb clonus, and tonic hindlimb extension in aminoglutethimide-pretreated mice.

There was a significant effect of antagonist treatment on PTZ dose required for myoclonic jerk ($F(2,53) = 6.25$, $p < 0.01$). Overall, RU38486 decreased PTZ doses required for this convulsion sign relative to spironolactone ($p < 0.01$).

Figure 8. Effects of corticosteroid receptor antagonists plus 0 or 1 mg/kg corticosterone on PTZ-induced myoclonic jerk and face and forelimb clonus in mice pretreated with 25 mg/kg aminoglutethimide. Bars represent means ± SEM. (* significantly different from 0 corticosterone - Vehicle group, + significantly different from 1 mg/kg corticosterone - Vehicle group).

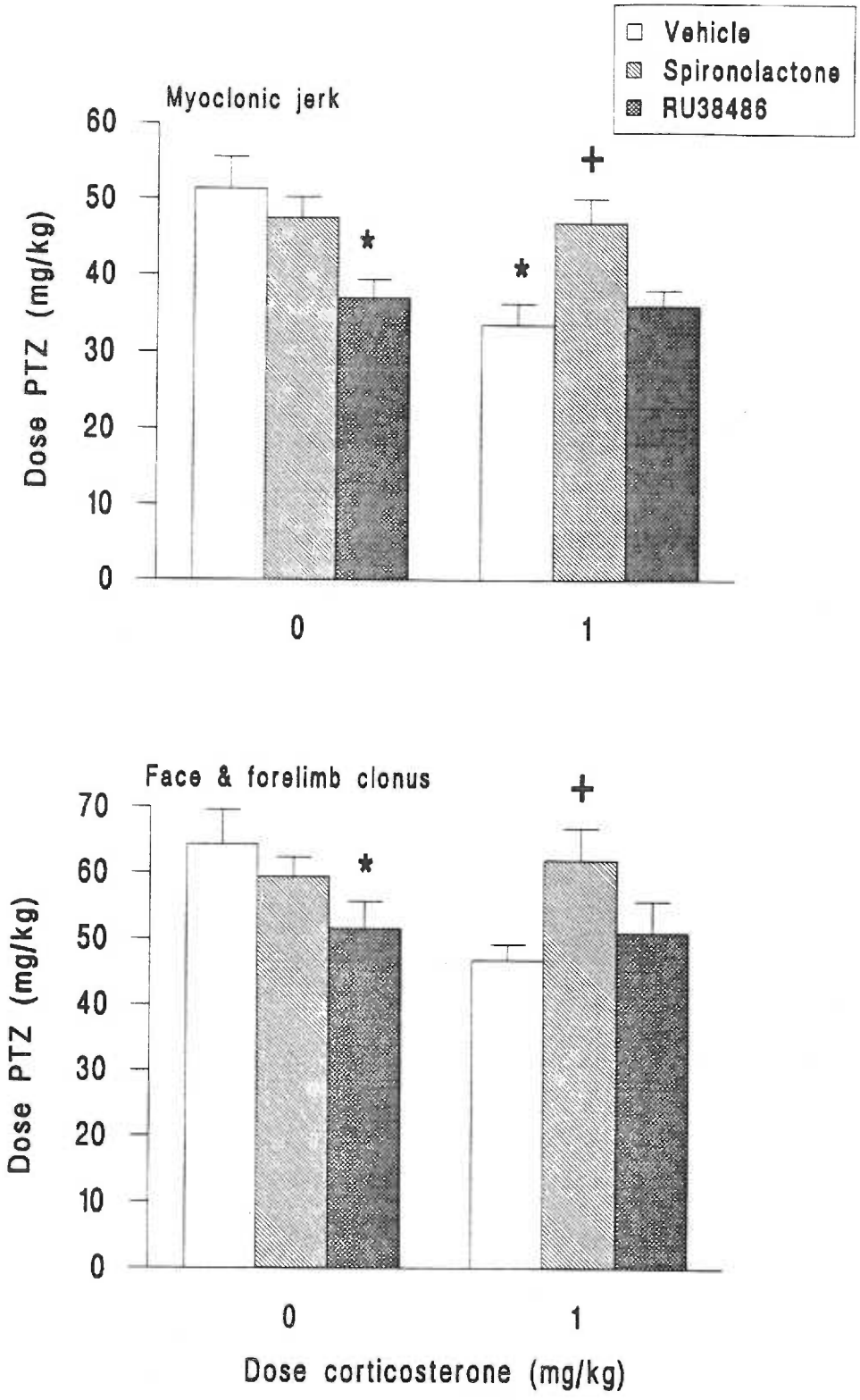
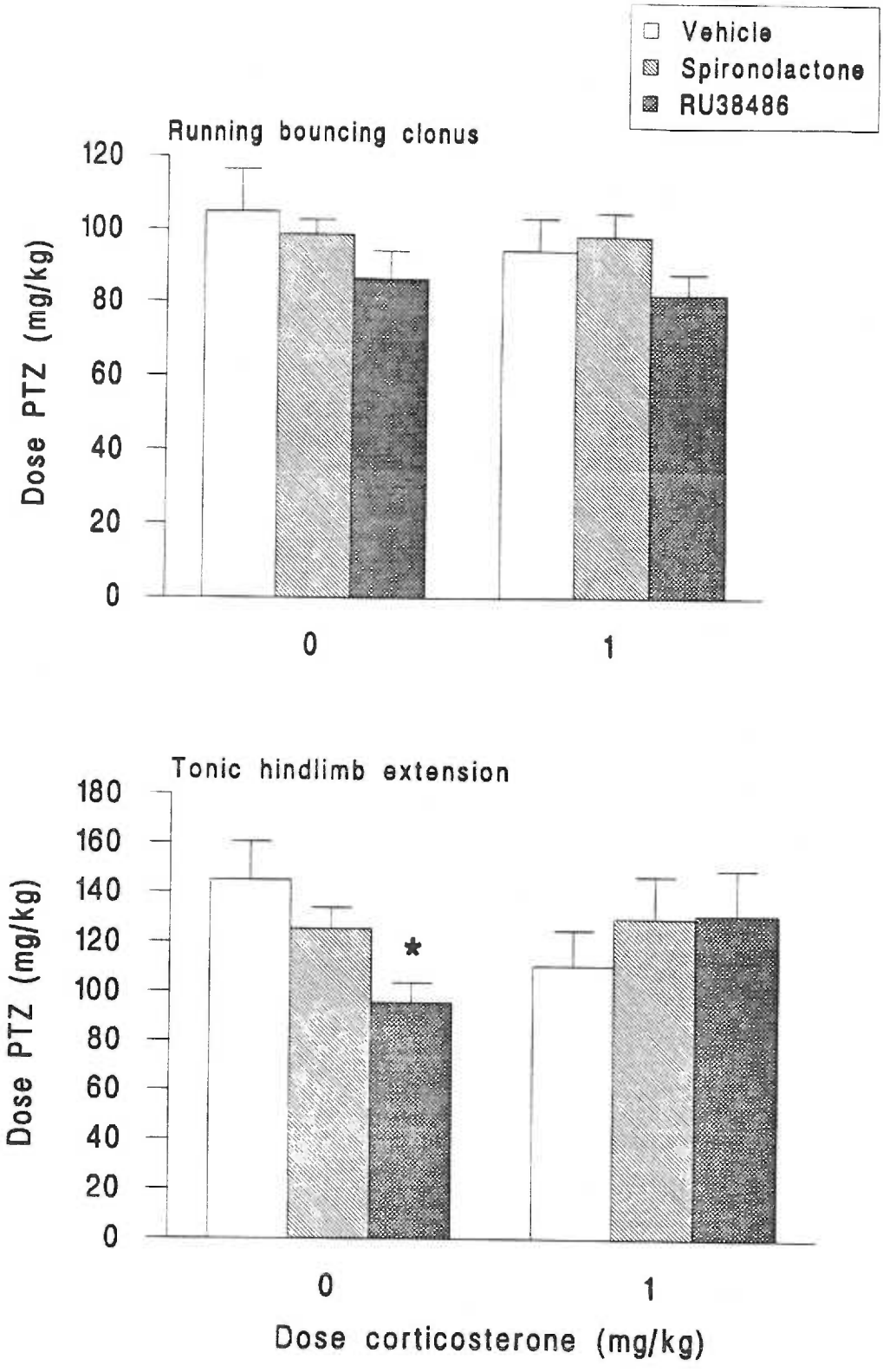

Vehicle
Spironolactone
RU38486
Myoclonic jerk
Dose PTZ (mg/kg)
60
50
40
30
20
10
0
*
*
+
0
1
Face & forelimb clonus
Dose PTZ (mg/kg)
70
60
50
40
30
20
10
0
*
+
0
1
Dose corticosterone (mg/kg)

Figure 9. Effects of corticosteroid receptor antagonists plus 0 or 1 mg/kg corticosterone on PTZ-induced running bouncing clonus and tonic hindlimb extension in mice pretreated with 25 mg/kg aminoglutethimide. Bars represent means ± SEM. (* significantly different from 0 corticosterone - Vehicle group).


Vehicle
Spironolactone
RU38486
Running bouncing clonus
Dose PTZ (mg/kg)
120
100
80
60
40
20
0
0
1
Tonic hindlimb extension
Dose PTZ (mg/kg)
180
160
140
120
100
80
60
40
20
0
*
0
1
Dose corticosterone (mg/kg)

Corticosterone also significantly affected myoclonic jerk ($F(1,53) = 8.36$, $p < 0.01$). Overall, corticosterone decreased the PTZ dose required for this convulsion sign. Antagonist treatment did not significantly affect face and forelimb clonus ($F(2,53) = 2.54$, $p = 0.09$), running bouncing clonus ($F(2,53) = 2.36$, $p = 0.10$), or tonic hindlimb extension ($F(2,53) = 0.60$, $p = 0.55$). Corticosterone also had no significant effects on face and forelimb clonus ($F(1,53) = 3.22$, $p = 0.08$), running bouncing clonus ($F(1,53) = 0.24$, $p = 0.78$), and tonic hindlimb extension ($F(1,53) = 0$, $p = 0.99$).

There was a significant interaction between antagonist and corticosterone treatments for PTZ doses required for myoclonic jerk ($F(2,53) = 5.83$, $p < 0.01$), face and forelimb clonus ($F(2,53) = 3.72$, $p < 0.05$), and tonic hindlimb extension ($F(2,53) = 3.26$, $p < 0.05$). Simple main effect analyses for myoclonic jerk revealed that corticosterone significantly decreased the dose of PTZ required in vehicle-treated mice ($F(1,53) = 19.99$, $p < 0.001$), but did not affect doses required in antagonist-treated mice. In addition, these analyses revealed an effect of antagonist treatment within the 0 mg/kg corticosterone group ($F(2,53) = 6.16$, $p < 0.01$) and the 1 mg/kg group ($F(2,53) = 5.96$, $p < 0.01$). Further comparisons indicated that RU38486 decreased PTZ doses required in the 0 mg/kg corticosterone group ($p < 0.025$) and that spironolactone increased PTZ doses required in the 1 mg/kg corticosterone group ($p < 0.025$). Simple

main effect analyses for face and forelimb clonus also revealed that corticosterone decreased doses of PTZ required in vehicle-treated mice ($F(1,53) = 10.46$, $p < 0.01$). In addition, there was a significant effect of antagonist treatment in mice that received 1 mg/kg corticosterone ($F(2,53) = 3.8$, $p < 0.05$). Further analysis indicated that spironolactone increased PTZ doses over those required in vehicle-treated mice ($p < 0.025$). Simple main effect analyses for tonic hindlimb extension revealed a significant effect of corticosterone in mice treated with RU38486 ($F(1,53) = 4.68$, $p < 0.05$). In addition, there was a significant effect of antagonist treatment in 0 mg/kg corticosterone groups ($F(2,53) = 3.21$, $p < 0.05$). RU38486 significantly decreased PTZ doses relative to vehicle ($p < 0.025$).

Plasma corticosterone levels obtained in this experiment are shown in Table 6. Levels were significantly increased by corticosterone treatment ($F(1,53) = 251.14$, $p < 0.0001$), but were unaffected by antagonist treatment.

IV.B. Strychnine

Experiment 6:

The purpose of Experiment 6 was to examine the effects of corticosterone on strychnine-induced convulsions. The results of this experiment are shown in Table 7. These results suggested that strychnine-induced convulsions were

Table 6. Plasma corticosterone concentrations (µg/dl) following PTZ-induced convulsions in mice pretreated with 25 mg/kg aminoglutethimide plus corticosterone and/or receptor antagonists.

Corticosterone (mg/kg)	Vehicle	Spironolactone	RU38486
0	2.9 ± 0.7	2.8 ± 0.62	2.4 ± 0.6
1	25.7 ± 2.8	27.3 ± 2.2	26.1 ± 2.3

Plasma concentrations are expressed as means ± SEM. There was an significant overall effect of corticosterone dose on plasma corticosterone levels (1 mg/kg > 0 mg/kg, $p < 0.0001$).

insensitive to modulation by corticosterone, at least at the doses administered in this experiment.

There was no effect of any of the corticosterone doses tested on doses of strychnine required for myoclonic jerk ($F(3,45) = 1.96$, $p = 0.13$), running bouncing clonus ($F(3,45) = 2.51$, $p = 0.07$), or tonic hindlimb extension ($F(3,45) = 1.79$, $p = 0.16$).

Plasma corticosterone levels achieved in this experiment are also shown in Table 7. There was a significant effect of corticosterone dose on plasma hormone levels ($F(3,45) = 40.92$, $p < 0.0001$). Each dose of corticosterone significantly increased plasma levels over each smaller dose ($p < 0.05$).

Experiment 7:

The purpose of Experiment 7 was to examine the effects of type I and type II corticosteroid receptor antagonists on strychnine-induced convulsions. The results of this experiment are shown in Table 8. These results suggested that type I and type II corticosteroid receptors are not involved in modulating strychnine-induced convulsions.

There were no effects of antagonist treatment on strychnine-induced myoclonic jerk ($F(2,16) = 0.15$, $p = 0.86$), running bouncing clonus ($F(2,16) = 0.01$, $p = 0.99$), or tonic hindlimb extension ($F(2,16) = 0.03$, $p = 0.97$). In addition, there was no effect of antagonist treatment on plasma corticosterone levels ($F(2,16) = 0.43$, $p = 0.66$).

Table 7. Doses of strychnine (mg/kg) required for convulsions and plasma corticosterone levels in mice following corticosterone preinjection.

Corticosterone (mg/kg)	Myoclonic jerk	Running bouncing clonus	Tonic hindlimb extension	Plasma corticosterone (µg/dl)
0	0.95 ± 0.06	1.15 ± 0.04	1.23 ± 0.03	3.37 ± 0.65
0.5	0.86 ± 0.07	1.09 ± 0.07	1.18 ± 0.07	8.66 ± 0.82*
1	1.09 ± 0.06	1.28 ± 0.05	1.35 ± 0.06	12.03 ± 1.09*
5	0.96 ± 0.05	1.25 ± 0.04	1.31 ± 0.05	20.99 ± 1.91*

Strychnine doses are expressed as means ± SEM. There was a significant effect of corticosterone dose on plasma corticosterone levels. * Significantly different from 0 mg/kg corticosterone ($p < 0.01$).

Table 8. Doses of strychnine required for convulsions (mg/kg) and plasma corticosterone levels (µg/dl) in mice following treatment with vehicle, 5 mg/kg spironolactone, or 5 mg/kg RU38486.

Drug	Myoclonic jerk	Running bouncing clonus	Tonic hindlimb extension	Plasma corticosterone (µg/dl)
Vehicle	1.14 ± 0.04	1.34 ± 0.13	1.42 ± 0.13	2.2 ± 0.9
Spironolactone	1.10 ± 0.07	1.33 ± 0.10	1.39 ± 0.10	3.0 ± 0.9
RU38486	1.15 ± 0.08	1.32 ± 0.05	1.38 ± 0.05	1.9 ± 0.5

Strychnine doses and plasma corticosterone concentrations are expressed as means ± SEM. There were no significant effects of corticosteroid receptor antagonist treatment on any of these measures.

Experiment 8:

The purpose of Experiment 8 was to examine the effects of aminoglutethimide on strychnine-induced convulsions. The results of this experiment are shown in Figure 10. There were no effects of aminoglutethimide on strychnine-induced myoclonic jerk ($F(4,18) = 0.47$, $p = 0.76$), running bouncing clonus ($F(4,18) = 0.21$, $p = 0.93$), or tonic hindlimb extension ($F(4,18) = 0.47$, $p = 0.76$).

Plasma corticosterone levels achieved in this experiment are shown in Figure 11. There was a significant effect of aminoglutethimide on plasma corticosterone levels ($F(4,18) = 11.04$, $p < 0.001$). All doses of aminoglutethimide significantly reduced plasma hormone levels relative to 0 mg/kg ($P < 0.01$). Further experiments with strychnine were not performed, as these convulsions did not appear to be sensitive to either increases or decreases in plasma corticosterone levels.

IV.C. Kainic acid

Experiment 9:

The purpose of Experiment 9 was to examine the effects of corticosterone on convulsions elicited by kainic acid. The results of this experiment are shown in Table 9. These results suggested that kainic acid-induced convulsions were sensitive to the proconvulsant effects of corticosterone, as corticosterone decreased doses of kainic acid required for convulsions.

Figure 10. Dose-response effects of aminoglutethimide on strychnine-induced convulsions. Symbols represent means ± SEM.

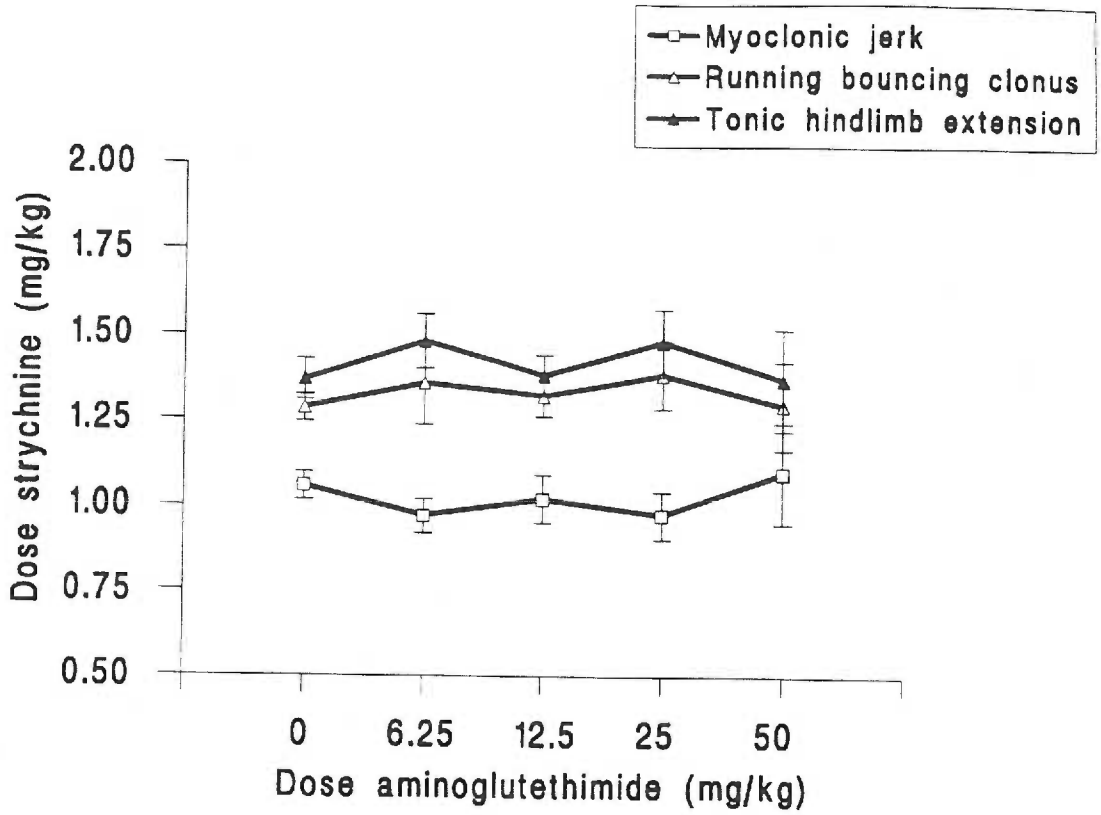

Myoclonic jerk
Running bouncing clonus
Tonic hindlimb extension
Dose strychnine (mg/kg)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0
6.25
12.5
25
50
Dose aminoglutethimide (mg/kg)

Figure 11. Dose-response effects of aminoglutethimide on plasma corticosterone levels. Blood was sampled following strychnine-induced convulsions. Symbols represent means ± SEM. Non-visible error bars indicate SEM less than symbol size.

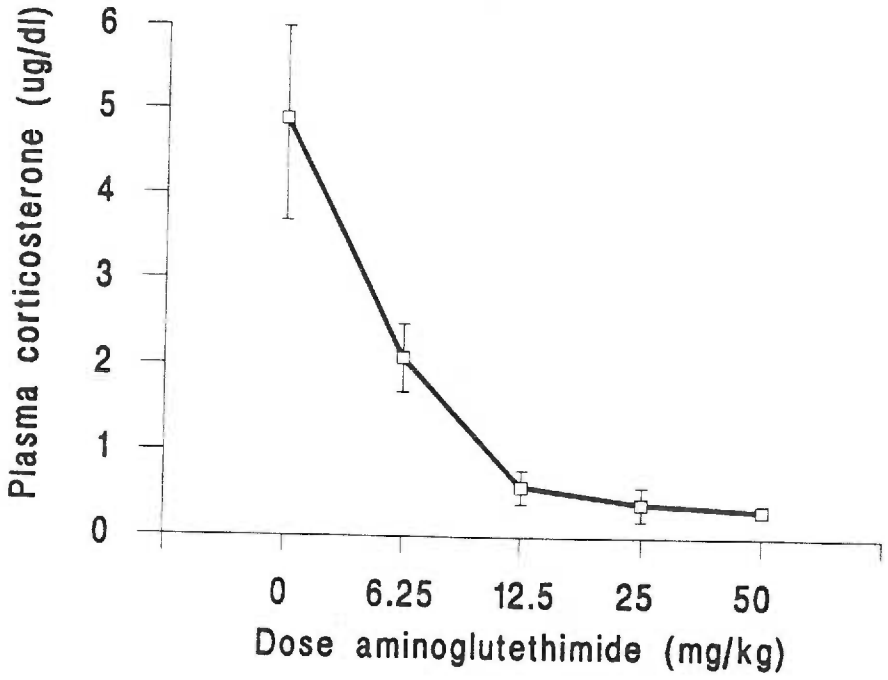

Plasma corticosterone (ug/dl)
6
5
4
3
2
1
0
0
6.25
12.5
25
50
Dose aminoglutethimide (mg/kg)

Table 9. Doses of kainic acid (mg/kg) required for convulsions and plasma corticosterone levels in mice following corticosterone preinjection.

Corticosterone (mg/kg)	Wild running clonus	Tonic hindlimb extension	Plasma corticosterone (µg/dl)
0	162.3 ± 14.4	192.1 ± 16.4	5.8 ± 0.8
0.5	140.9 ± 11.5	175.4 ± 12.9	13.9 ± 1.7*
1	89.4 ± 5.8*	123.4 ± 8.9*	16.4 ± 1.6*
5	116.8 ± 11.4*	147.6 ± 12.5	34.5 ± 2.3*

Kainic acid doses are expressed as means ± SEM. There were significant effects of corticosterone dose on dose of kainic acid required for wild running clonus and tonic hindlimb extension ($p < 0.01$). In addition, there was a significant effect of corticosterone dose on plasma corticosterone levels ($p < 0.001$). * Significantly different from 0 mg/kg corticosterone ($p < 0.05$).

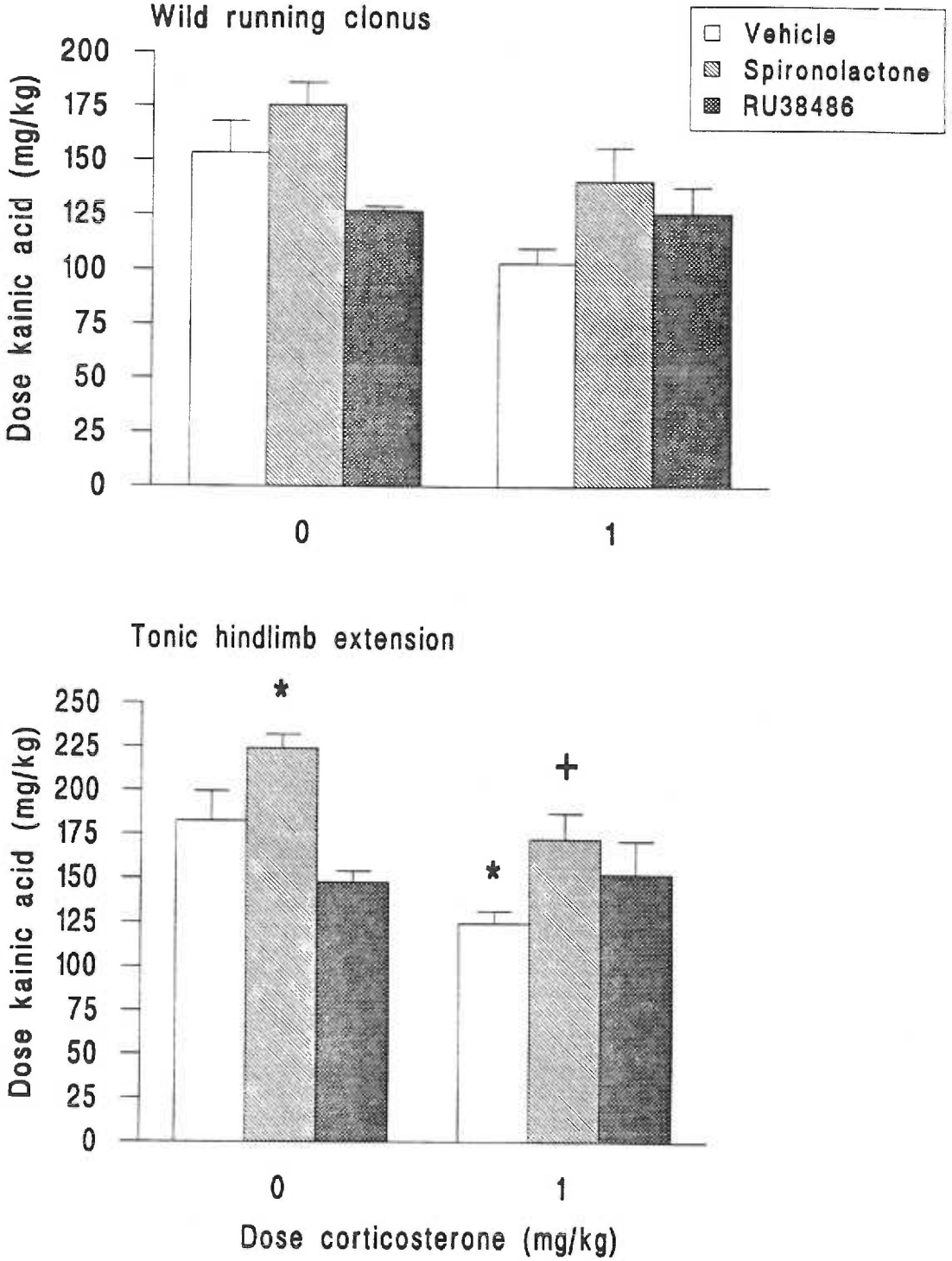
There was a significant effect of corticosterone on doses of kainic acid required for wild running clonus ($F(3,15) = 7.84$, $p < 0.01$) and tonic hindlimb extension ($F(3,15) = 5.42$, $p < 0.01$). Doses of kainic acid required for wild running clonus were significantly decreased by 1 ($p < 0.01$) and 5 ($p < 0.05$) mg/kg corticosterone. Doses of kainic acid required for tonic hindlimb extension were significantly decreased by 1 mg/kg corticosterone ($p < 0.05$).

Plasma corticosterone levels determined following tonic hindlimb extension are also shown in Table 9. There was a significant effect of corticosterone on plasma levels of this hormone ($F(3,15) = 47.99$, $p < 0.0001$). All doses of corticosterone significantly increased plasma corticosterone levels relative to 0 mg/kg ($p < 0.01$).

Experiment 10:

The purpose of Experiment 10 was to determine whether the proconvulsant effect of 1 mg/kg corticosterone on kainic acid-induced convulsions was reversed by spironolactone (type I antagonist) or RU38486 (type II antagonist). The results of this experiment are shown in Figure 12. These results suggested that spironolactone had anticonvulsant action upon kainic acid-induced convulsions. Because spironolactone had an effect in the 0 mg/kg corticosterone group, it is difficult to determine whether its reversing action in the 1 mg/kg corticosterone group was due to

Figure 12. Effects of corticosteroid receptor antagonists on kainic acid-induced convulsions in mice treated with 0 or 1 mg/kg corticosterone. Bars represent means ± SEM. (* significantly different from 0 corticosterone - Vehicle group, + significantly different from 0 corticosterone - Spironolactone group and 1 mg/kg corticosterone - Vehicle group).


Wild running clonus
Vehicle
Spironolactone
RU38486
Dose kainic acid (mg/kg)
200
175
150
125
100
75
50
25
0
0
1
Tonic hindlimb extension
*
+
*
250
225
200
175
150
125
100
75
50
25
0
0
1
Dose corticosterone (mg/kg)

pharmacological antagonism or simply due to behavioral antagonism. At any rate, it is clear that spironolactone exerted moderate anticonvulsant action, while RU38486 did not reverse the corticosterone effect and may even possess proconvulsant activity.

There was a significant main effect of antagonist treatment on doses of kainic acid required for wild running clonus ($F(2,29) = 4.16$, $p < 0.05$) and tonic hindlimb extension ($F(2,29) = 7.55$, $p < 0.01$). Overall, spironolactone treatment increased doses of kainic acid required for these convulsion signs ($p < 0.05$). Corticosterone significantly decreased doses of kainic acid required for wild running clonus ($F(1,29) = 10.74$, $p < 0.01$) and tonic hindlimb extension ($F(1,29) = 12.92$, $p < 0.01$).

There was a moderately significant interaction between antagonist and corticosterone treatments on kainic acid-induced tonic hindlimb extension ($F(2,29) = 3.26$, $p = 0.05$). Simple main effects analysis of this interaction revealed a significant effect of corticosterone in mice treated with vehicle ($F(1,29) = 11.00$, $p < 0.01$) and spironolactone ($F(1,29) = 7.48$, $p < 0.025$), but not RU38486. In addition, the results of these analyses revealed a significant effect of antagonist treatment in both the 0 mg/kg corticosterone group ($F(2,29) = 8.21$, $p < 0.001$) and the 1 mg/kg corticosterone group ($F(3.62) = p < 0.05$). Further analysis indicated that spironolactone increased doses of kainic acid required in mice treated with 0 mg/kg ($p < 0.05$) and 1 mg/kg

($p < 0.025$) corticosterone over those of vehicle-treated mice. The interaction between corticosterone and antagonist treatment was not significant for wild running clonus ($F(2,29) = 2.32$, $p = 0.12$).

Plasma corticosterone levels achieved in this experiment are shown in Table 10. There was a significant main effect of corticosterone on plasma levels of this hormone ($F(1,29) = 69.58$, $p < 0.0001$). There was no effect of antagonist treatment, however.

Experiment 11:

The purpose of Experiment 11 was to examine the effects of aminoglutethimide on kainic acid-induced convulsions. The results of the experiment are shown in Figure 13. These results indicated that kainic acid-induced convulsions were sensitive to the anticonvulsant effects of aminoglutethimide.

There were significant effects of aminoglutethimide on doses of kainic acid required for wild running clonus ($F(4,18) = 3.34$, $p < 0.05$) and tonic hindlimb extension ($F(4,18) = 3.26$, $p < 0.05$). Only 50 mg/kg aminoglutethimide significantly increased doses of kainic acid necessary for these convulsions ($p < 0.05$).

Plasma corticosterone levels achieved in this experiment are shown in Figure 14. There was a significant effect of aminoglutethimide on plasma corticosterone levels ($F(4,18) = 18.52$, $p < 0.0001$). The three highest doses of

Table 10. Effects of corticosteroid antagonists ± corticosterone on plasma corticosterone levels (µg/dl) following kainic acid-induced convulsions in mice .

Corticosterone (mg/kg)	Vehicle	Spironolactone	RU38486
0	8.1 ± 1.4	8.3 ± 1.9	8.3 ± 0.7
1	20.8 ± 2.4	18.8 ± 1.2	16.8 ± 0.6

Corticosterone levels are expressed as means ± SEM. There was a significant overall effect of corticosterone on plasma levels ($p < 0.0001$), but no effect of treatment with antagonist.

Figure 13. Dose-response effects of aminoglutethimide on kainic acid-induced convulsions. Symbols represent means ± SEM.

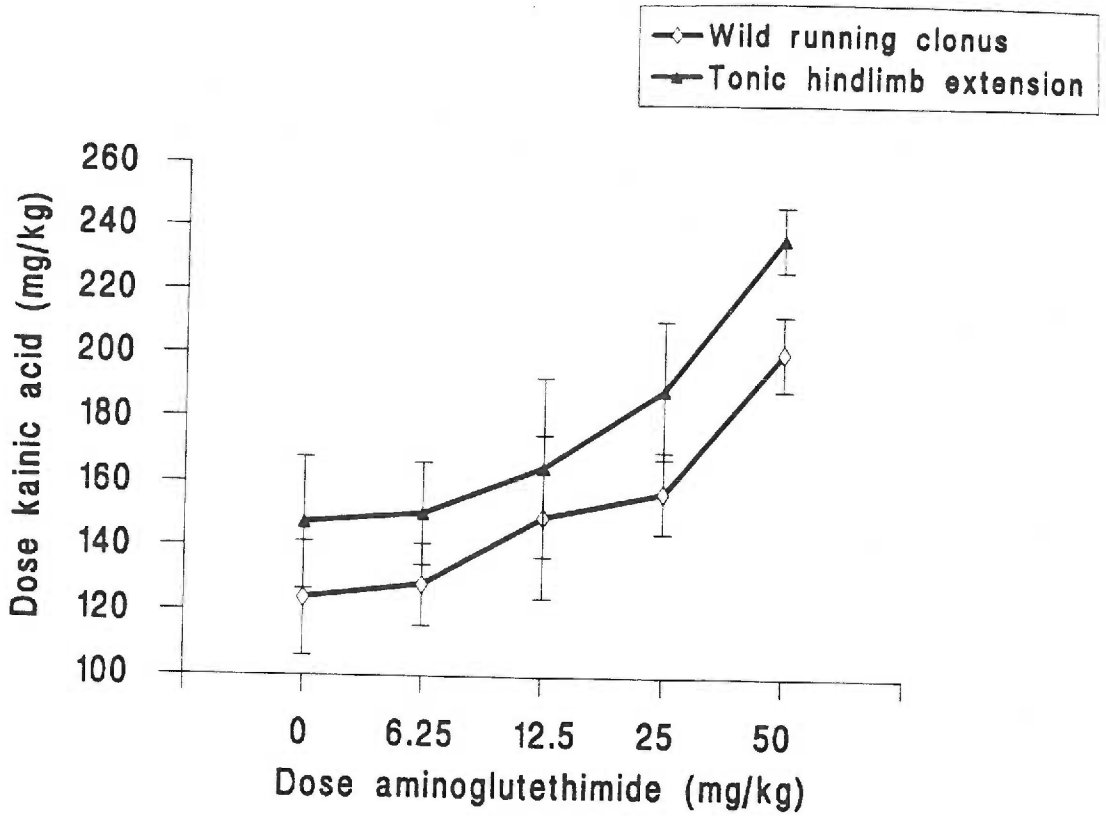
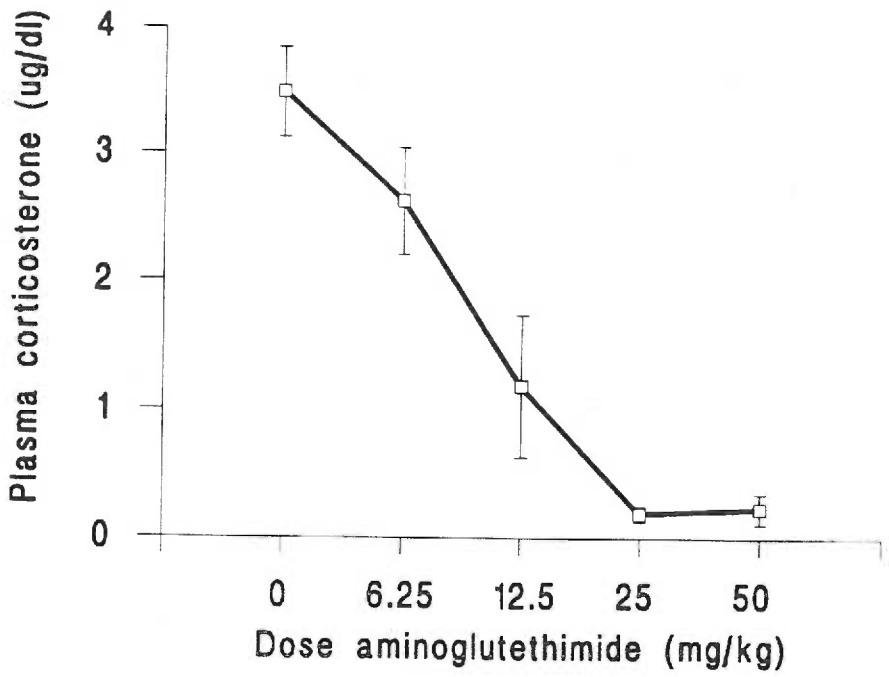

Wild running clonus
Tonic hindlimb extension
Dose kainic acid (mg/kg)
260
240
220
200
180
160
140
120
100
0
6.25
12.5
25
50
Dose aminoglutethimide (mg/kg)

Figure 14. Dose-response effects of aminoglutethimide on plasma corticosterone levels. Blood was sampled following kainic acid-induced convulsions. Symbols represent means ± SEM.


Plasma corticosterone (ug/dl)
4
3
2
1
0
0
6.25
12.5
25
50
Dose aminoglutethimide (mg/kg)

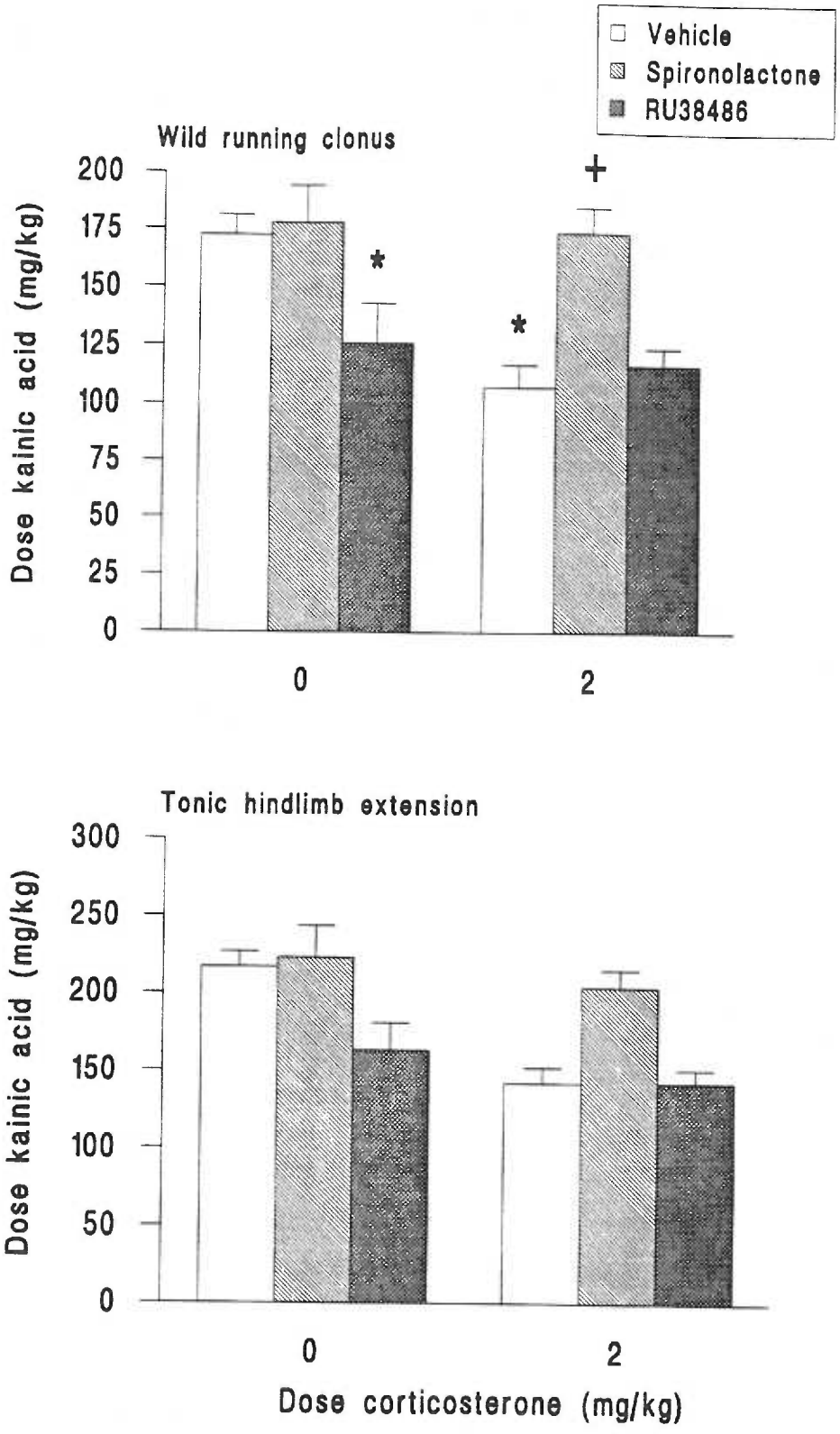
aminoglutethimide (12.5, 25, and 50 mg/kg) significantly decreased plasma hormone levels over the 0 and 6.25 mg/kg doses ($p < 0.01$).

Experiment 12:

The purpose of Experiment 12 was to examine the effects of spironolactone (type I antagonist) and RU38486 (type II antagonist) on the reversing effect of 2 mg/kg corticosterone on kainic acid-induced convulsions in aminoglutethimide-pretreated mice. The results of this experiment are shown in Figure 15. These results indicated that corticosterone decreased doses of kainic acid required for wild running clonus in mice pretreated with aminoglutethimide, but not antagonist. This corticosterone effect was not present in spironolactone-treated mice. This suggests that the proconvulsant effect of corticosterone was mediated via action at type I receptors. Although not significant, a similar result was observed with kainic acid-induced tonic hindlimb extension. RU38486 appeared to have a moderate proconvulsant effect upon these convulsions.

There was a significant main effect of antagonist treatment on kainic acid-induced wild running clonus ($F(2,30) = 10.34$, $p < 0.001$) and tonic hindlimb extension ($F(2,30) = 9.88$, $p < 0.001$). Overall, spironolactone-treated mice required higher doses of kainic acid for convulsions and RU38486-treated mice required less kainic

Figure 15. Effects of corticosteroid receptor antagonists plus 0 or 2 mg/kg corticosterone on kainic acid-induced wild running clonus and tonic hindlimb extension in mice pretreated with 25 mg/kg aminoglutethimide. Bars represent means ± SEM. (* significantly different from 0 corticosterone - Vehicle group, + significantly different from 2 mg/kg corticosterone - Vehicle group).


Vehicle
Spironolactone
RU38486
Wild running clonus
Dose kainic acid (mg/kg)
200
175
150
125
100
75
50
25
0
*
*
+
0
2
Tonic hindlimb extension
Dose kainic acid (mg/kg)
300
250
200
150
100
50
0
0
2
Dose corticosterone (mg/kg)

acid for convulsions. There was also a significant main effect of corticosterone treatment on wild running clonus ($F(1,30) = 7.82$, $p < 0.01$) and tonic hindlimb extension ($F(1,30) = 12.73$, $p < 0.01$). Overall, corticosterone decreased the dose of kainic acid required for convulsions.

The interaction between antagonist and corticosterone treatments was significant for wild running clonus ($F(2,30) = 4.15$, $p < 0.05$), but just missed significance for tonic hindlimb extension ($F(2,30) = 2.84$, $p = 0.07$). Simple main effects analysis for wild running clonus revealed significant effects of antagonist treatment in mice treated with 0 mg/kg corticosterone ($F(2,30) = 5.69$, $p < 0.01$) and 2 mg/kg corticosterone ($F(2,30) = 9.45$, $p < 0.001$). RU38486 decreased the dose of kainic acid required for wild running clonus in mice treated with 0 mg/kg corticosterone ($p < 0.01$). Spironolactone increased the dose of kainic acid required for wild running clonus in mice treated with 2 mg/kg corticosterone ($p < 0.01$). There was also a significant simple main effect of corticosterone in the vehicle-treated mice ($F(1,30) = 15.91$, $p < 0.01$).

Plasma corticosterone levels obtained in this experiment are shown in Table 11. Two mg/kg corticosterone significantly increased plasma levels in mice pretreated with aminoglutethimide relative to those receiving no corticosterone ($F(1,30) = 141.52$, $p < 0.0001$). There was no effect of antagonist treatment ($F(2,30) = 2.23$, $p = 0.12$)

Table 11. Effects of corticosteroid antagonists ± corticosterone on plasma corticosterone levels (µg/dl) following kainic acid-induced convulsions in mice pretreated with 50 mg/kg aminoglutethimide.

Corticosterone (mg/kg)	Vehicle	Spironolactone	RU38486
0	0.12 ± 0.08	0.13 ± 0.04	0.11 ± 0.04
2	23.1 ± 3.3	14.7 ± 2.1	20.8 ± 2.9

Corticosterone levels are expressed as means ± SEM. There was a significant overall effect of corticosterone on plasma levels ($p < 0.0001$), but no effect of treatment with antagonist.

and the interaction between corticosterone and antagonist treatments was not significant ($F(2,30) = 2.38$, $p = 0.11$).

V. DISCUSSION

The experiments presented above were aimed at testing the hypothesis that type I receptors mediate excitatory and type II receptors mediate inhibitory effects of corticosteroids. The results are summarized in Table 12. Excitatory effects of corticosterone were observed in several instances. These excitatory effects were mediated by action at type I receptors. Most convulsion types affected by type I manipulations are believed to involve limbic structures, therefore these data support a modulatory role for these receptors upon hippocampal excitability. No inhibitory effects within the dose range tested were seen. The type II antagonist, RU38486, produced interesting, but unexpected results. These will be discussed later in this section.

The dose of PTZ required for myoclonic jerk was decreased by 0.5 mg/kg corticosterone. Plasma corticosterone levels of these mice were approximately 12 µg/dl, consistent with increased type I binding (as determined in rat hippocampal cytosol; Reul and de Kloet, 1985). Spironolactone blocked this effect of corticosterone, further implicating type I receptors in mediating the excitatory effect of corticosterone.

Table 12. Summary of the effects of corticosteroid manipulations on susceptibility to convulsions produced by (A) PTZ, (B) Strychnine, and (C) Kainic acid.

Symbols:
- 0 no change in convulsion susceptibility relative to VEH-VEH group
- \- decreased convulsion susceptibility (increased dose of convulsant required) relative to VEH-VEH group
- \+ increased convulsion susceptibility (decreased dose of convulsant required) relative to VEH-VEH group

A. PTZ

	VEH	SPIR	RU38486
VEH	0	0/−	0/+
CORT (0.5 mg/kg)	+	0	0/+
AMG (25 mg/kg)	−	−	0/+
CORT (1 mg/kg) + AMG (25 mg/kg)	0	−	0/+

B. Strychnine

	VEH	SPIR	RU38486
VEH	0	0	0
CORT	0	not tested	not tested
AMG	0	not tested	not tested

C. Kainic acid

	VEH	SPIR	RU38486
VEH	0	−	0/+
CORT (1 mg/kg)	+	0	0/+
AMG (50 mg/kg)	−	−	0/+
CORT (2 mg/kg) + AMG (50 mg/kg)	0	−	0/+

Furthermore, corticosterone reversed the anticonvulsant effect of aminoglutethimide on PTZ-induced convulsions. Again, the levels achieved (about 17 µg/dl) were believed to maximize type I binding. Spironolactone inhibited the reversing effect of corticosterone on PTZ-induced myoclonic jerk, face and forelimb clonus, and tonic hindlimb extension in mice pretreated with aminoglutethimide. Running bouncing clonus was unaffected by any of the presently tested corticosteroid manipulations, except RU38486 which had a general proconvulsant effect on PTZ-induced convulsions.

There was some inconsistency concerning the effects of the corticosteroid manipulations on each PTZ-induced convulsion sign. For example, face and forelimb clonus and tonic hindlimb extension were not affected by corticosterone alone, but corticosterone was able to attenuate the anticonvulsant effect of aminoglutethimide on these signs. In Experiment 3, tonic hindlimb extension was not affected by aminoglutethimide, however group sizes were smaller in this experiment than the others employing aminoglutethimide (Experiments 4 and 5). It appeared that corticosterone exerted a permissive effect upon PTZ-induced face and forelimb clonus and tonic hindlimb extension. Although exogenously administered corticosterone had no effect on these convulsions, endogenous corticosterone acting via type I receptors was apparently necessary for normal expression of these convulsion signs. PTZ-induced myoclonic jerk was also sensitive to type I modulation, however running

bouncing clonus appeared to be insensitive to corticosteroid manipulation.

Corticosterone, producing plasma levels of 8-21 µg/dl, had no effect on strychnine-induced convulsions. Decreasing corticosterone levels with aminoglutethimide also did not affect these convulsions. Furthermore, treatment with spironolactone or RU38486 did not alter doses of this convulsant required for convulsions. These results suggested that strychnine-induced convulsions are corticosteroid-independent.

In contrast, kainic acid-induced convulsions were sensitive to corticosteroid manipulations. One mg/kg corticosterone (producing plasma levels of approximately 16 µg/dl) significantly decreased doses of kainic acid required for wild running clonus and tonic hindlimb extension. Spironolactone had an anticonvulsant effect itself and reversed the proconvulsant effect of corticosterone. Aminoglutethimide significantly increased doses of kainic acid required for convulsions. Corticosterone reversed this effect of aminoglutethimide on wild running clonus; this reversal was blocked by spironolactone. As was the case with PTZ-induced convulsions, RU38486 appeared to have a proconvulsant effect over kainic acid-induced convulsions, even in aminoglutethimide-treated mice.

These experiments support an excitatory role of corticosterone via type I receptor action over several convulsion types. Interestingly, not all convulsions were

affected in the same way by the same corticosteroid manipulations. This suggests that corticosteroid effects over nervous system excitability may have neuroanatomical and/or neurochemical specificity.

Several PTZ-induced convulsions were sensitive to modulation by type I corticosteroid receptors. Because PTZ is believed to produce convulsions through its action at the $GABA_A$ receptor complex, it is possible that activated type I receptors modulate this neurotransmitter system. For example, it was shown that adrenalectomy decreased the affinity of the GABA receptor complex for the synthetic agonist, muscimol in several rat brain areas (Majewska, Bisserbe and Eskay, 1985). This effect was reversed by corticosterone, but not dexamethasone, suggesting it may be mediated via type I receptor activation. However, in this case, decreased corticosterone levels were associated with increases in neuronal excitability, as decreased GABA binding would potentially result in attenuated chloride flux. In another report, this group found effects of corticosteroids on binding of t-[35S]Butyl bicyclophosphorothionate (TBPS) to the convulsant site on the GABA receptor complex (Majewska, 1987a). Increases in TBPS binding were found one hour following exposure to low concentrations of cortisol, whereas high cortisol concentrations decreased TBPS binding. This effect was not believed to be due to a direct interaction of cortisol with the convulsant site, but due to some sort of allosteric

modulation. In another study, adrenalectomy was found to increase numbers of benzodiazepine binding sites in several mouse brain regions (Miller, Greenblatt, Barnhill, Thompson and Shaderh, 1988). It is possible that adrenalectomy could result in enhanced chloride flux through the GABA receptor ionophore. In this report, the effect of adrenalectomy was reversed by chronic treatment with the mineralocorticoid, deoxycorticosterone, but not by the glucocorticoid, dexamethasone, suggesting a role of the type I receptor in this effect.

It is likely, however, that the effects of corticosteroid manipulations on convulsions found in the present experiments were not entirely dependent on global effects on the GABAergic system. If corticosteroid manipulations primarily were affecting the GABAergic system, PTZ-induced running bouncing clonus would be expected to be affected in a similar manner as the other PTZ-induced convulsions. In addition, GABA is believed to be involved to some degree in a wide variety of convulsions, as it has been suggested that GABA projections from the substantia nigra modulate seizures originating in both forebrain and brainstem regions (Gale, 1988). If this system were affected globally by the present corticosteroid manipulations, then strychnine-induced convulsions and PTZ-induced running bouncing clonus would be expected to be affected. Finally, although effects on TBPS binding mentioned above were rapid, the effects on muscimol and

benzodiazepine binding were observed two weeks and one week, respectively, following adrenalectomy. It is unclear whether acute alterations in corticosteroid levels would have these same effects.

Kainic acid-induced convulsions appeared to be quite sensitive to manipulations in type I receptor binding. It is possible that activated type I receptors modulate glutamatergic systems. Chronic exposure to high corticosterone levels exacerbated levels of glutamate and aspartate in rat hippocampus during kainic acid-induced seizures (Stein-Behrens, Elliot, Miller, Schilling, Newcombe and Sapolsky, 1992). This enhanced excitotoxicity by glucocorticoids is believed in be mediated by type II corticosteroid receptors (Packan and Sapolsky, 1990). In another study, transient manipulation of corticosterone levels did not significantly alter kainic acid binding in hippocampus (Clark and Cotman, 1992). Unless PTZ-induced convulsions are sensitive to excitatory amino acid inputs (which they certainly may be) this may not be a principal effect of type I receptors on nervous system excitability.

Because type I receptors are localized primarily in the hippocampus, they may modulate neuronal transmission specifically in this structure. For example, it has been suggested that type I receptor-mediated events may include enhanced excitatory amino acid neurotransmission (Joëls and de Kloet, 1992) and decreased inhibition by serotonin (Joëls, Hesen and de Kloet, 1991) in the hippocampus.

Serotonin, via activation of 5-HT_{1A} receptors, increases Ca^{2+}-independent K^+ conductances, thus causing cell hyperpolarization. It has been postulated that corticosterone, via activation of type I receptors, inhibits the coupling of the 5-HT_{1A} to its target G protein. In addition, it is possible that there are specific effects of corticosteroids, via activation of type I receptors, upon GABAergic neurotransmission in the hippocampus.

Forebrain regions have been implicated in mediating face and forelimb clonus as well as kainic acid-induced convulsions. Indeed, PTZ-induced face and forelimb clonus and kainic acid-induced convulsions were sensitive to type I corticosteroid receptor manipulations in the present experiments. Strychnine-induced convulsions and PTZ-induced running bouncing clonus, which are believed to originate primarily in the brain stem and spinal cord were insensitive to the corticosteroid manipulations presently used. This suggests that corticosteroids, via activation of type I receptors, modulate hippocampal excitability.

However, PTZ-induced tonic hindlimb extension was sensitive to type I corticosteroid receptor modulation. This convulsion sign is proposed to originate in brainstem regions. It is possible that alterations in hippocampal excitability may lead to alterations in the excitability of other brain areas, however PTZ-induced running bouncing clonus and strychnine-induced convulsions, other supposed brainstem convulsions, were insensitive to type I

corticosteroid receptor modulation. Perhaps PTZ-induced tonic hindlimb extensor convulsions are specifically modulated by hippocampal projections. Alternatively, type I corticosteroid receptors may affect neurotransmission outside the limbic system.

No inhibitory effects of corticosterone were observed in the present experiments. There are several potential reasons for this outcome. One possibility is that the doses of corticosterone administered were too low to observe an effect. Perhaps the degree of type II binding was insufficient for a pronounced inhibitory effect. However, the plasma corticosterone levels achieved following the highest corticosterone dose were well within the range observed during states of stress. In addition, pilot studies revealed no effects of 10 and 20 mg/kg corticosterone on PTZ-induced convulsions, despite plasma hormone levels of approximately 40 and 70 µg/dl, respectively. Alternatively, the time following corticosterone treatment may have been too long or too short to optimize inhibitory effects. For example, it has been suggested that type II-mediated effects are slower to develop than type I effects (de Kloet, Joëls, Oitzl and Sutanto, 1991), therefore perhaps given more time following corticosterone, inhibitory effects would have been observed. Finally, perhaps the excitatory type I effects obscured the expression of inhibitory type II effects. Type I receptors are fully occupied when type II receptors are being bound.

Indeed, the highest dose of corticosterone did not affect PTZ-induced convulsions and affected kainic acid-induced convulsions to a lesser degree than the next lowest dose. If this were the case then it would be expected that RU38486 would remove the type II inhibition produced by the high dose of corticosterone, resulting in an excitatory effect. This was not tested in the present experiments, as low doses of corticosterone, not believed to fully occupy type II receptors, were used in experiments employing the antagonists.

The results with RU38486 were unexpected. RU38486 had moderate, but significant proconvulsant action over PTZ- and kainic acid-induced convulsions. This is the predicted direction of response based on the hypothesis that type II receptors mediate inhibitory effects of corticosteroids. However, plasma corticosterone levels in the experiments utilizing this compound were relatively low, thus the type II receptor was expected to be mostly in its unbound form. In addition, the progesterone receptor, also a target for the antagonistic effect of RU38486, would be expected to be in its unbound form. In this case there should be no effect of antagonist treatment. This should have been true especially in the experiments in which mice were pretreated with aminoglutethimide (plasma levels below 1 µg/dl). The results of these experiments suggest that RU38486 has an effect on nervous system excitability which is independent of its steroid receptor antagonistic properties.

It is possible that RU38486 has agonistic actions in the absence of appropriate ligand. It was shown that RU38486 given alone decreased population spikes recorded from rat hippocampal slices (Rey, Carlier and Soumireu-Mourat, 1989). This effect was in the same direction as the effects of high concentrations of corticosterone, therefore it was suggested that RU38486 may have agonist properties. This is possible, as activated antihormone-receptor complexes have been detected (Beck et al., 1993). However, if RU38486 had agonistic activity in the present studies, the results are not supportive of an inhibitory effect of type II receptor activation.

It is possible that RU38486 acts directly on some neurotransmitter system to alter neuronal excitability. For example, several neurosteroids have been identified which bind directly to the $GABA_A$ receptor complex and either increase or decrease the ability of the channel to conduct chloride ions (Majewska, 1992). It remains to be determined whether RU38486 has any direct GABAergic effect or whether it affects another neurotransmitter/ ion channel system involved in nervous system excitability. In any case, it was interesting that this compound consistently decreased doses of PTZ and kainic acid required for convulsions, but did not alter doses of strychnine required for convulsions.

Although the results of these experiments clearly suggest a role for type I receptors in mediating excitatory effects of corticosteroids on various convulsion types,

there are several alternative explanations for the results. It is possible that the effects of corticosterone and the antagonists were mediated via peripheral mechanisms. Perhaps corticosterone had general metabolic effects or even specific effects on motor systems which altered convulsion susceptibility. Or perhaps corticosterone or its receptor antagonists affected the absorption of the convulsants into the nervous system. For example, it is possible that RU38486 displayed proconvulsant effects because it increased the absorption or distribution of PTZ and kainic acid in the brain. While these issue cannot be entirely ruled out, the fact that the corticosteroid effects were not generalized across every convulsant and every convulsion sign suggests a more specific mechanism of action.

VI. CONCLUSIONS

The results of the present experiments support the hypothesis that corticosteroids, via activation of type I receptors, increase nervous system excitability. This effect is not generalizable across a wide range of convulsion types, but shows specificity to limbic convulsions which may relate to the anatomical localization of type I corticosteroid receptors and/or the specific neurotransmitter system(s) affected by corticosteroids. Postulated type II corticosteroid receptor effects were not observed in the present experiments. RU38486 did have proconvulsant action, but because it was effective even when

little type II binding was possible, it is believed that its effects were independent of antagonistic action at type II receptors.

These experiments were important for several reasons. First, the same corticosteroid manipulations were used for each convulsion examined, therefore results from each experiment can be discussed in relationship to each other. Second, several different measures of nervous system excitability were used in order to examine the specificity of corticosteroid effects. Third, these experiments were performed on intact living animals, therefore they potentially have more direct clinical relevance than experiments performed on brain slices.

The results of these experiments are significant for several reasons. For example, it may be predicted that convulsions which are affected in a similar manner by corticosteroid manipulations may share common underlying neurochemical and/or neuroanatomical pathways. In the model of seizures proposed by Gale (1988), it was argued that certain propagation pathways may function as common denominators for the development of a variety of convulsive disorders. The results of the present experiments suggest that convulsions which are limbic in nature are modulated by activity of type I corticosteroid receptors.

Another significant contribution of these data is the possible clinical utility of type I corticosteroid receptor antagonists as anticonvulsants. The treatment of complex

partial (limbic) seizures has been problematic for clinicians. It is the least likely form of epilepsy to be sufficiently treated with the available anticonvulsants (Fisher, 1989). Perhaps type I corticosteroid receptor antagonists, which presumably have greatest action in the limbic system, will prove to be useful for treatment of this form of epilepsy.

Finally, these data are important within the field of stress research. They support the concept of homeostasis through corticosteroid receptor balance first discussed by Selye (1946) and furthered by de Kloet, Joëls, Oitzl and Sutanto (1991). This hypothesis states that a balance in type I- and type II- mediated responses to corticosteroids is of critical importance for the setpoint of disease susceptibility. A relative deficiency or excess of type I- over type II- mediated actions is proposed to alter the setpoint of homeostatic control and lead to conditions of reduced or enhanced responsiveness to neurotransmitters, impairments in behavioral adaptation, and promotion of disease susceptibility. Indeed, in the present experiments, alterations in type I binding led to altered susceptibility to convulsions.

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