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Pharmacology, Biochemistry and Behavior 78 (2004) 483–493

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Androgens in the hippocampus can alter, and be altered by, ictal activity

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Received 2 February 2004; received in revised form 9 March 2004; accepted 13 April 2004

Abstract

Steroid hormones, such as androgens, can modulate seizure processes. This review summarizes prior research and presents new data that support the role of androgens in modulating seizure processes. Testosterone, the primary endogenous androgen, has antiseizure effects in people and in animal models of epilepsy. Furthermore, testosterone's antiseizure effects may involve actions of its 5α-reduced metabolite and neuroactive steroid, 5α-androstane-3α,17β-diol (3α-diol). The hippocampus is a target for androgen action and is involved in many types of seizure disorder. Data suggest that actions of androgens in the hippocampus may be important for androgens' antiseizure effects. Interestingly, there may also be a reciprocal relationship between androgens and seizures. Ictal activity can alter the gonadal responsiveness of people with epilepsy and in animal models of seizure disorder. Thus, this paper will review data in support of androgens' antiseizure effects. Further understanding of androgens' role in seizure processes is important for potential therapeutic effects.

Keywords: Seizures; 3α-diol; Pentylenetetrazole; Animal model

1. Introduction

More than 50 million people worldwide suffer from epilepsy, with over 125,000 new cases diagnosed each year in the United States (Satzinger, 1994). Unfortunately, traditional antiepileptic drug therapies are only effective at controlling seizures in about 70% of those who have seizure disorder. Traditional antiepileptic drugs target GABA and glutamate systems, which are major gatekeeper systems in the brain. Thus, it is not surprising that these traditional therapies are often associated with numerous negative side effects. Notably, steroid hormones can also have actions via GABA (Majewska et al., 1986) and glutamate (Avoli and Olivier, 1987), and there is evidence that steroids can influence seizure processes.

This review will discuss recent research from our laboratory, which suggests that androgens in the hippocampus may alter, and be altered by, seizures. First, background information from clinical studies will be presented, followed by sex differences reported in rodent models and effects of extirpation and replacement of androgens on seizure activity of rodents. The variable effects of androgen metabolites on ictal activity are also discussed. Second, an overview and rationale of the pentylenetetrazole (PTZ) seizure model that we utilize is provided. Finally, data from our laboratory is presented, which suggest the following. (1) Endogenous life-span variations in androgen levels are associated with altered seizure susceptibility of male rats. (2) Proximate androgen levels can mediate seizures of young-adult and aged male rats. (3) Peripheral administration of testosterone and its 5α-reduced metabolites, dihydryotestosterone and 5α-androstane-3α,17β-diol (3α-diol), has similar effects to reduce the ictal activity of gonadectomized (GDX) young-adult male rats. (4) Testosterone, dihydrotestosterone, and 3α-diol to the hippocampus of GDX young-adult male rats decrease seizure activity compared with vehicle administration. (5) Blocking testosterone's 5α-reduction attenuates its protective effects in the hippocampus. (6) 5α-reductase activity in the hippocampus is decreased following seizures. Together, these data suggest that androgens can have antiseizure effects in the PTZ model and these effects may be due, in part, to actions of 3α-diol in the hippocampus.

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doi:10.1016/j.pbb.2004.04.020

1.1. Androgens' modulation of seizures in men

The ictal activity of men with or without seizure disorder can be altered by testosterone, the primary androgen secreted by the gonads. Men with lower circulating concentrations of testosterone have a greater incidence of alcohol-withdrawal-induced seizures compared with men with higher levels of testosterone (Ruusa and Bergman, 1996). Testosterone administration decreased the number of seizures experienced by a young man with posttraumatic seizures compared with the number experienced prior to beginning testosterone treatment (Tan and Tan, 2001). The synthetic androgen, testenat, decreases the number and severity of epileptic seizures (Badalian et al., 1991). As well, testosterone treatment to men with seizure disorder can decrease seizure frequency (Herzog et al., 1998). These data suggest that androgens may play an important role in the seizure processes of men.

1.2. Androgens' modulation of seizures in rodent models

Data from rodent models also support a role of androgens in mediating ictal activity. There are sex differences in ictal activity that favor males. Male, compared with female, rodents are typically less susceptible to various types of seizures. Males have a higher seizure threshold for electroshock-induced seizures (Kokka et al., 1992). Young-adult males are also less sensitive to picrotoxin-induced seizures than are age-matched female rats (Pericic et al., 1996). The incidence of flumazenil-induced seizures is lower among male mice than that seen in female mice (Pesce et al., 1994). Male rats have fewer allylglycine-induced focal and generalized seizures compared with females (Thomas and Yang, 1991).

More direct evidence for androgens' modulation of seizures comes from extirpation and replacement studies in rodent models of seizure disorder. Removal of the primary endogenous source of androgens, the testes, attenuates the sex difference in ictal activity that typically favors males. GDX increases picrotoxin-, strychnine-, and PTZ-induced seizures (Pericic et al., 1996, Pesce et al., 2000; Thomas and McLean, 1991), and, in some cases, testosterone replacement reverses this effect (Frye and Reed, 1998; Pesce et al., 2000; Schwartz-Giblin et al., 1989). Together, these data suggest that androgens can have a protective role in seizure processes.

Although the above data suggest that androgens can have antiseizure effects, it should be noted that the role of androgens in seizure processes is not clear-cut. Indeed, in some reports, testosterone has proconvulsant effects. For example, testosterone significantly lowers the threshold for electroshock-induced seizures in intact male rats (Timiras and Hill, 1980). Androgens may have their excitatory effects due to the actions of testosterone itself, or its aromatized or 5α-reduced metabolites, estradiol and dihydrotestosterone, respectively. Estradiol and dihydrotestosterone can alter hippocampal excitability and function (Diamond et al., 1996; Herzog, 1999; Meyer and Gruol, 1994). As well, estradiol, testosterone, and dihydrotestosterone can enhance the development of amygdala-kindled seizures (Edwards et al., 1999). However, in this report, estradiol and testosterone had more salient effects to increase amygdala-kindled seizures than did dihydrotestosterone (a nonaromatizable androgen), and the administration of an aromatase inhibitor to intact male rats increased afterdischarge thresholds compared with vehicle administration (Edwards et al., 1999). This suggests that the proconvulsant effects of testosterone may be due to its aromatized products. In addition, as reviewed above, androgens can decrease seizures of male rats induced by picrotoxin, kainic acid, PTZ, and perforant pathway stimulation (Frye and Reed, 1998; Rhodes et al., 2004; Thomas and McLean, 1991). Thus, androgens' varied effects on ictal activity may be due to several factors, including the androgen regimen utilized (which may affect the ratio of aromatized to 5α-reduced metabolites), the model system, and/or the method of inducing seizures.

1.3. The seizure models that we have utilized

In our laboratory, we have used two pharmacological models of seizure disorder. Both kainic acid and PTZ have been used in our laboratory as models of generalized seizure disorder. Kainic acid is a rigid analog of the excitatory neurotransmitter, glutamate (McGeer et al., 1978), and can induce seizures in dosages less than those required to produce cell damage (Lothman et al., 1981). Although we began using kainic acid in our laboratory as a model of generalized seizure disorder (Frye and Bayon, 1998, 1999a,b; Frye and Reed, 1998; Frye and Scalise, 2000), we switched from using kainic acid to PTZ several years ago due to problems with the reliability and availability of kainic acid. As a result, all of the behavioral data from our laboratory presented in this review were obtained utilizing the PTZ model.

PTZ is a tetrazol derivative with consistent convulsive actions in several animal models (Stone, 1970). The seizure progression produced with the PTZ regimen that we utilize (70 mg/kg ip) is reliably observed and quantified using the scale devised by Racine (1972). PTZ initially produces myoclonic jerks (characterized by mouth and facial movements and head nodding), which then become sustained and typically lead to generalized tonic-clonic seizures (Huot and Radouco-Thomas, 1973). Thus, the behavioral data from our laboratory presented in this review are the incidence of myoclonus observed in a 10-min period following PTZ administration.

Our research on androgens' mediation of seizure activity is an extension of our ongoing research on progestins' effects and mechanisms to modulate ictal activity. Although we have only examined androgens' effects on the ictal activity of males using the PTZ model, we have observed a similar pattern of effects of androgens to protect against

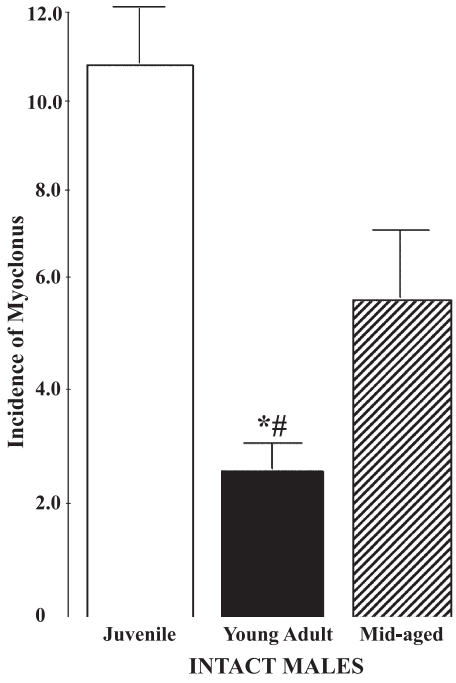

Fig. 1. Incidence of PTZ-induced (70 mg/kg) myoclonus of intact juvenile (open bar, $n=12$), young-adult (black bar, $n=12$), and mid-aged (striped bar, $n=12$) males. * Significantly different from juvenile males (Fishers Least Significant Difference Test, $P<.05$). # Tendency for difference from mid-aged males (Fishers Least Significant Difference Test, $P<.10$).

perforant pathway stimulation- and kainic acid-induced seizures in female rats (Frye and Reed, 1998). Furthermore, all of our findings of progestins' antiseizure effects have been observed between and within models. Progestins consistently decrease seizure activity induced by perforant pathway stimulation, kainic acid, or PTZ (Frye, 1995; Frye and Bayon, 1998, 1999a,b; Frye and Scalise, 2000; Frye and Muscatiello, 2001; Frye et al., 1998, 2000, 2002). As well, the patterns of progestin's effects on myoclonus, tonic-clonic seizures, and survival rates are similar. Thus, the incidence of myoclonus is presented in the present review as a reliable index of seizure activity.

1.4. Androgens effects on PTZ-induced seizures

Although sex differences in the ictal activity of adult rodents have been well investigated, other developmental differences that may influence ictal activity have not. Variations in endogenous androgens across the life span may also influence the ictal activity of rodents. Androgen levels of male rats begin to decline during middle-age (13–15 months old), and the most robust differences between intact young-adult (3–4 months old) and aged rats occur when aged rats are 24+ months old (Gray, 1978; Chambers and Phoenix, 1984). If androgens modulate seizures, then age-related changes in androgens would be expected to be associated with differences in ictal activity.

We have begun to examine the changes in ictal activity across the life span of male rats. Our data suggest that age-associated decreases in androgen levels occur concomitant with increased PTZ-induced seizures in aged rats (24 months old; Rhodes et al., 2004). Very young or mid-aged rats are more susceptible to seizures than are young-adult males. Intact, young-adult male rats (4 months old) have significantly fewer PTZ-induced (70 mg/kg ip) myoclonic seizures than do intact prepubertal juvenile (26 days old, randomly selected from multiple litters) and tend to have fewer myoclonic seizures than do the mid-aged (13-month-old) males (Fig. 1). These variations in seizures across the life span may be related to the differences in many age-related factors, one of which is androgen levels. To investigate this, androgen levels in the hippocampus were measured (per previous methods, Frye and Bayon, 1999a,b,c). Young-adult males have higher levels of testosterone, and its 5α-reduced metabolites, dihydrotestosterone, and 3α-diol, in the hippocampus than do juvenile rats, with the lowest levels, or mid-aged males, with intermediate levels (Table 1). Although opposite patterns of effects are observed for androgen levels in the hippocampus and seizures, which suggests that testosterone's 5α-reduced metabolites may mediate antiseizure effects in this model, questions remain. Whether the intermediate effects seen in mid-aged males were associated with absolute levels of androgens and/or prior exposure to androgens was not dissociated. As well, whether these antiseizure effects of androgens are causal was not demonstrated.

We have addressed the capacity of mid-aged rats to respond to androgen administration. Replacing back androgens to mid-aged rats reverses the effects of aging on ictal activity. The incidence of myoclonus of mid-aged rats (13 months old) administered with 3α-diol (1 mg/kg sc) is significantly decreased compared with mid-aged males administered with vehicle and similar to that of intact young-adult (4 months old) males (Fig. 2). Thus, proximate androgen levels can mediate ictal activity, independent of age. However, the effects of other developmental factors on seizures need to be ascertained.

Data from our laboratory suggest that proximate androgen levels or replacement can mediate androgens' antiseizure effects and that GDX is a valid model that produces similar effects on ictal activity as does aging. GDX young-adult rats (4 months) have a similar incidence of myoclonus as do intact aged males (24 months). The administration of testosterone to GDX young-adult males or intact senescent rats decreases the incidence of myoclonus in both groups to levels that are similar with intact young-adult males (Fig. 3). These data suggest that testosterone can have antiseizure

Table 1
Hippocampal T, DHT, and 3α-diol levels of intact juvenile, young-adult, and mid-aged male rats

Condition	T (ng/g)	DHT (ng/g)	3α-diol (ng/g)
Juvenile	0.5 ± 0.1	1.0 ± 0.2	0.2 ± 0.1
Young adult	4.0 ± 0.4	1.1 ± 0.1	2.6 ± 0.3
Mid-aged	2.7 ± 0.9	0.8 ± 0.2	1.3 ± 0.9

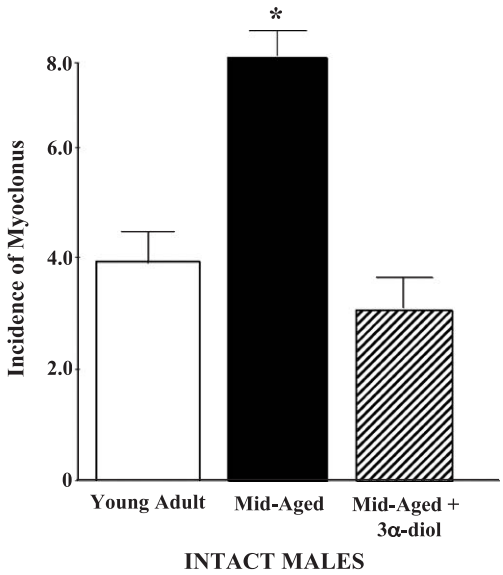

Fig. 2. Incidence of PTZ-induced (70 mg/kg) myoclonus of intact young-adult (open bar, $n=10$), mid-aged (black bar, $n=8$), or mid-aged males administered 3α-diol (striped bar, $n=10$). * Significantly different from all other groups (Fishers Least Significant Difference Test $P<.05$).

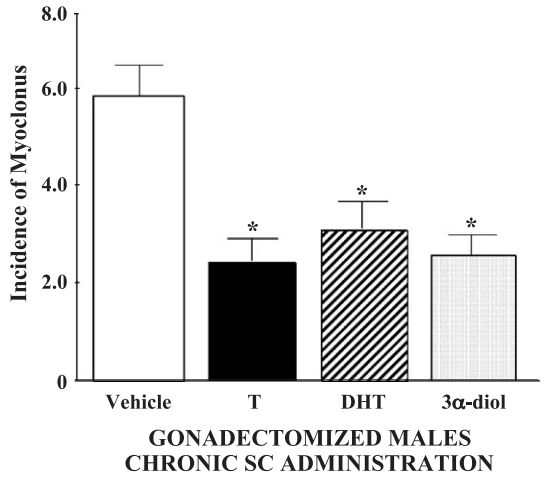

Fig. 4. Incidence of PTZ-induced (70 mg/kg) myoclonus of GDX males administered with chronic, systemic vehicle (open bar, $n=10$), T (black bar, $n=10$), DHT (striped bar, $n=10$), or 3α-diol (dotted bar, $n=10$). * Significantly different from vehicle-administered males (Fishers Least Significant Difference Test, $P<.05$).

effects but do not directly address the effects of testosterone's metabolites on ictal activity.

More recent data from our laboratory suggest that testosterone and its metabolites, dihydrotestosterone and 3α-diol, have similar effects to decrease PTZ-induced ictal activity. GDX young-adult male rats (4 months old) administered with testosterone, dihydrotestosterone, or 3α-diol have significantly fewer PTZ-induced (70 mg/kg ip) myoclonic seizures compared with vehicle-administered rats. This pattern of results is similar with acute (1mg/kg subcutaneous injections; Fig. 4) or chronic (1-week exposure to subcutaneous silastic implants, per previous methods, Frye and Seliga, 2001; Fig. 5) systemic androgen administration. The hippocampal androgen levels produced by these androgen regimens (Table 2) reveal a similar pattern of effects as is seen for plasma androgen levels (data not shown). Notably, the similar effects of testosterone, dihydrotestosterone, and 3α-diol on PTZ-induced seizures, together with increased 3α-diol in the hippocampus with each of the androgen regimen, suggest that androgens' antiseizure effects may be due, at least in part, to the actions of 3α-diol in the hippocampus.

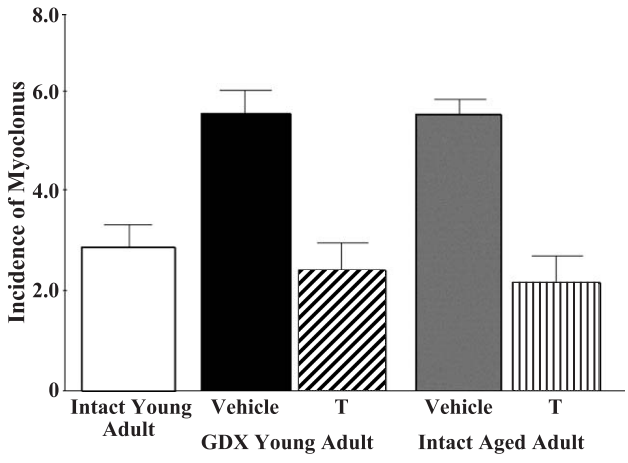

Fig. 3. Incidence of PTZ-induced (70 mg/kg) myoclonus of intact young-adult (open bar, $n=4$), GDX young-adult administered with vehicle (black bar, $n=4$) or testosterone (diagonally striped bar, $n=3$), or intact aged males administered with vehicle (grey bar, $n=3$) or testosterone (vertically striped bar, $n=3$).

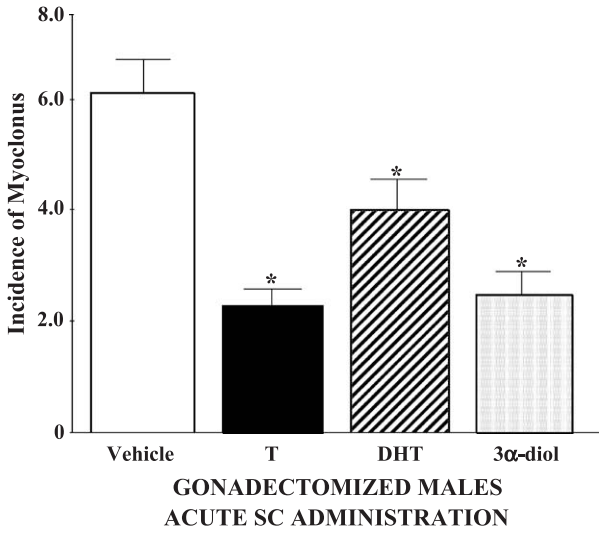

Fig. 5. Incidence of PTZ-induced (70 mg/kg) myoclonus of GDX males administered with acute, systemic vehicle (open bar, $n=12$), T (black bar, $n=12$), DHT (striped bar, $n=12$), or 3α-diol (dotted bar, $n=12$). * Significantly different from vehicle-administered males (Fishers Least Significant Difference Test, $P<.05$).

Table 2
Hippocampal T, DHT, and 3α-diol levels of rats administered with vehicle, T, DHT, or 3α-diol via silastic capsules or subcutaneous injections

		T (ng/g)	DHT (ng/g)	3α-diol (ng/g)
Silastic capsules	Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	T	0.2 ± 0.1	0.4 ± 0.2	0.9 ± 0.1
	DHT	0.0 ± 0.0	1.0 ± 0.1	0.5 ± 0.1
	3α-diol	0.0 ± 0.0	0.8 ± 0.1	1.0 ± 0.1
Subcutaneous injections (1 mg/kg)	Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	T	1.8 ± 0.3	0.6 ± 0.1	4.8 ± 1.6
	DHT	0.0 ± 0.0	0.8 ± 0.4	1.1 ± 0.3
	3α-diol	0.0 ± 0.0	0.5 ± 0.1	3.3 ± 1.1

1.5. Androgens' actions in the hippocampus for antiseizure effects

The hippocampus is a site of androgens' actions. First, tritiated testosterone is taken up in the hippocampus of rats (Pfaff, 1968). Second, there is an abundance of androgen-receptor-expressing cells in the rat hippocampus (Brown et al., 1995; Simerly et al., 1990). Third, all of the enzymes necessary for testosterone's metabolism, aromatase, 5α-reductase, and 3α-hydroxysteroid dehydrogenase, are located within the hippocampus (Li et al., 1997; Ivanova and Beyer, 2000; Jacobs et al., 1999). Fourth, there are sex differences in neuronal firing and synapse growth in the hippocampus and in the vulnerability of the hippocampus to damages that are attenuated by GDX and reinstated with androgen replacement. Notably, androgens reverse the effects of castration on these processes (Frye and McCormick, 2000a,b; Leranth et al., 2003; Pouliot et al., 1996; Smith et al., 2002). Thus, these data suggest that the hippocampus is a target for androgens' actions.

Androgens also have neuroprotective effects in the hippocampus. In vitro, testosterone can attenuate β-amyloid toxicity, oxidative stress-induced cell death, and serum-deprivation-induced cell death in hippocampal neurons (Ahlbom et al., 2000; Hammond et al., 2001). There is also support for androgens' neuroprotective effects in in vivo models. Androgens can reduce vulnerability to hippocampal damage induced by adrenalectomy or kainic acid (Frye and McCormick, 2000a,b; Ramsden et al., 2003). Indeed, data from our laboratory suggest that testosterone's protective effects may be due to its 5α-reduction, rather than aromatization. Testosterone, dihydrotestosterone, and 3α-diol similarly protect against adrenalectomy-induced cell death in the hippocampus (Frye and McCormick, 2000a,b). Furthermore, GDX male rats administered with testosterone had significantly fewer pyknotic cells in the hippocampus compared with the vehicle-administered rats. Coadministration of finasteride, a 5α-reductase inhibitor (Fig. 6), but not ATD, an aromatase inhibitor, attenuated testosterone's protective effects (Fig. 7). Although estrogen has well-known protective effects in this (Frye, 2001), and other neuroprotection models not involving seizures (estrogen typically has excitatory effects in seizure models), these data suggest

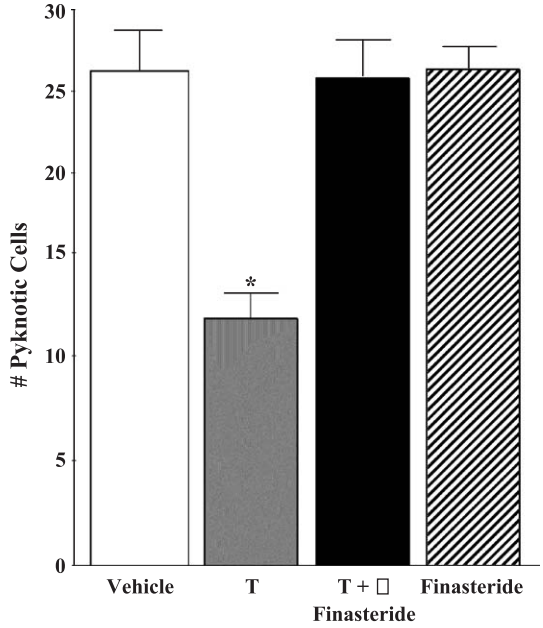

Fig. 6. Number of pyknotic cells in the dentate gyrus of GDX rats administered with vehicle (open bar, $n=25$), testosterone (grey bar, $n=22$), testosterone, and finasteride (black bar, $n=19$) or finasteride (striped bar, $n=19$). * Significantly different from all other groups (Fishers Least Significant Difference Test, $P<.05$).

that some of androgens' protective effects in the hippocampus may be due, in part, to actions of its 5α-reduced, rather than aromatized, metabolites. How these protective effects

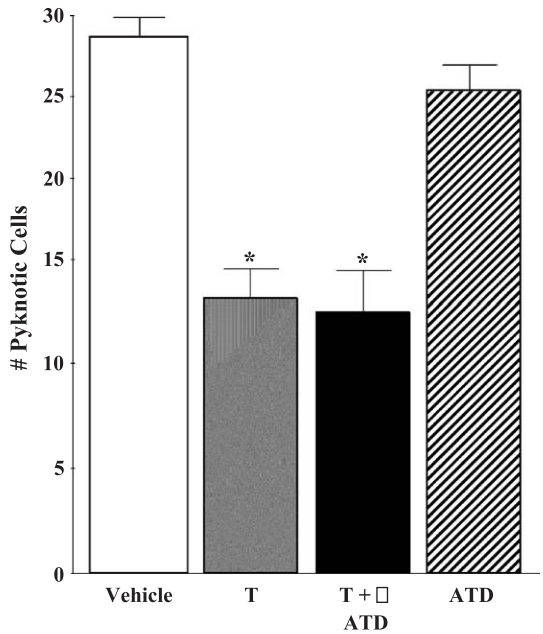

Fig. 7. Number of pyknotic cells in the dentate gyrus of GDX rats administered with vehicle (open bar, $n=25$), testosterone (grey bar, $n=22$), testosterone, and ATD (black bar, $n=20$), or ATD (striped bar, $n=20$). * Significantly different from vehicle and ATD groups (Fishers Least Significant Difference Test, $P<.05$).

of androgens in the hippocampus relate to ictal activity has not been established.

Ictal activity can have profound negative effects on the hippocampus and hippocampally-mediated processes. In animal models, hippocampal damage is reported following pilocarpine-, kainate-, PTZ-, and kindling-induced ictal activity (Hoffman et al., 2003; Pohle et al., 1997; Rigoulot et al., 2004). As well, performance in hippocampally-mediated learning tasks is disrupted following seizures. Declarative memory, which depends on a properly functioning hippocampus (Squire, 1992), is often disrupted in people with seizure disorder (Abrahams et al., 1999; Helmstaedter, 2002). In rodents, deficits in hippocampally-mediated spatial memory are associated with seizures (Collier and Routtenberg, 1984; Kelsey et al., 2000; Liu et al., 2003). Due to the functional consequences that can result from the vulnerability of the hippocampus to damage, further investigation of the protective and/or mnemonic effects of androgens, in this and other models, is important (Edinger et al., 2004).

The brain region(s) where androgens may have their actions to mediate ictal activity is not known. However, based on the data discussed above, the hippocampus is a logical site to begin to investigate. Systemic administration of testosterone, dihydrotestosterone, or 3α-diol similarly reduced ictal activity and increased levels of 3α-diol in the hippocampus compared with vehicle administration. The hippocampus is a target of androgens' actions and has all of the enzymes necessary for testosterone's metabolism to dihydrotestosterone and 3α-diol. Androgens can protect the hippocampus from damage. Finally, the hippocampus is implicated in several types of seizure processes. Thus, our investigations have begun to focus on the effects of androgens in the hippocampus for antiseizure effects.

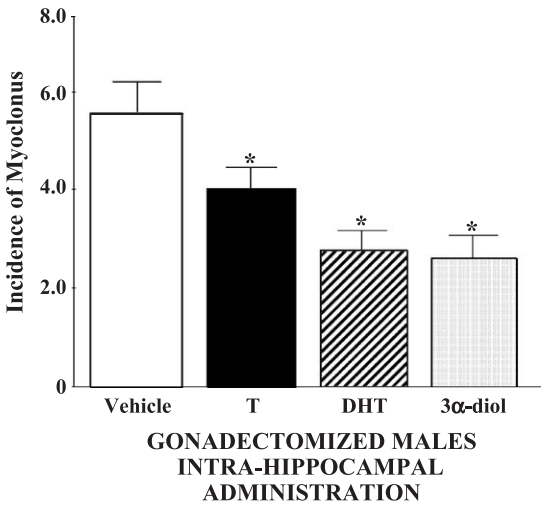

Fig. 8. Incidence of PTZ-induced (70 mg/kg) myoclonus of GDX males administered with vehicle (open bar, $n=12$), T (black bar, $n=12$), DHT (striped bar, $n=12$), or 3α-diol (dotted bar, $n=12$) to the hippocampus. * Significantly different from vehicle-administered males (Fishers Least Significant Difference Test, $P<.05$).

Table 3
Hippocampal T, DHT, and 3α-diol levels of rats administered with vehicle, T, DHT, or 3α-diol to the hippocampus

Condition	T (ng/g)	DHT (ng/g)	3α-diol (ng/g)
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T	0.4 ± 0.1	0.0 ± 0.0	1.9 ± 0.9
DHT	0.0 ± 0.0	3.6 ± 0.4	1.9 ± 0.2
3α-diol	0.0 ± 0.0	2.3 ± 0.5	0.8 ± 0.5

Androgens' antiseizure effects may be due to the actions of testosterone and its metabolites in the hippocampus. Thus, we have examined the capacity of the hippocampus to respond to direct androgen administration. Implants of crystalline testosterone, dihydrotestosterone, or 3α-diol to the dorsal hippocampus of GDX rats 2 h prior to PTZ-induced seizures significantly reduce the number of myoclonic seizures and increase androgen levels in the hippocampus, compared with that seen following vehicle administration (Fig. 8; Table 3). The reduced ictal activity seen following intrahippocampal administration of androgens is similar with that seen following systemic (acute or chronic) administration (Figs. 4 and 5). That direct implants of androgens or systemic androgen regimen have similar effects to decrease ictal activity may be due to the maximal effects produced by supraphysiological androgen regimen. Indeed, our investigations to this point have focused on androgen regimens that produce high, sustained androgen levels in the hippocampus. Whether there are dose- or exposure-dependent effects of androgens on ictal activity needs to be investigated.

2. Interim summary

The data discussed to this point support a role of 5α-reduced androgens in modulating the PTZ-induced ictal activity of male rats. First, there are sex differences that favor males, and these differences are attenuated by the removal of the primary endogenous source of androgen secretion, the testes. Second, there are developmental differences in the ictal activity of male rats. Very young or mid-aged rats, which have low androgen levels in the hippocampus, have increased ictal activity compared with young-adult males with higher hippocampal androgen levels. Finally, data suggest that androgens' antiseizure effects may be due, in part, to the actions of its metabolite, 3α-diol, in the hippocampus. Systemic and intrahippocampal testosterone, dihydrotestosterone, and 3α-diol all have similar effects to decrease seizures and increase 3α-diol in the hippocampus. Although these data suggest that the 5α-reduced metabolites of testosterone can reduce ictal activity in our model, other reports suggest that testosterone can have proconvulsant effects. One explanation for these differences may be that androgen levels are altered by seizures. This possibility is discussed below.

As discussed above, ictal activity can have profound effects on the hippocampus. Notably, hippocampally focused seizures can also disrupt the hypothalamic regulation of pituitary secretion, which can subsequently alter gonadal function (Drislane et al., 1994; Herzog, 1999; Herzog et al., 1986). Generalized and focal limbic seizures alter normal gonadal structure, physiology, and circulating androgen levels in male rats (Knobil, 1990). Thus, ictal activity in the hippocampus may influence androgen secretion.

2.1. Altered endocrine/reproductive profiles of men with epilepsy

Some men with epilepsy have a different androgen profile than do men with epilepsy. Among men with epilepsy, androgen deficiency is overrepresented (Herzog, 1999). Bioavailable testosterone is abnormally low among men with epilepsy compared with controls without epilepsy (Herzog et al., 2003). Also common among people with epilepsy are reproductive disorders (Herzog, 1999). Sexual interest, sexual responsiveness, and sexual function are unusually low among a majority of epileptic men compared with controls (Blumer, 1970; Herzog et al., 1986, 2003; Morrell, 1991; Morrell et al., 1994; Taylor, 1969). Men with epilepsy have only one third of the expected number of offspring compared with control populations. Hypogonadism, endocrine disorders, and reproductive dysfunction also are more common among women with epilepsy than among controls (Herzog, 1999).

The above data suggest that there are differences in the endocrine and reproductive functions of people with epilepsy compared with those without seizure disorder. However, the basis of these differences is not known. It may be that there are etiological differences among people with epilepsy that produce these comorbid effects on endocrine and reproductive function. It is also possible that seizures themselves may have effects to alter parameters of endocrine and reproductive function of men with epilepsy. Alternatively, other factors, such as antiepileptic drugs, may alter endocrine and reproductive functions. Indeed, enzyme-inducing antiepileptic drugs are thought to be involved in decreased androgen levels and sexual dysfunction among men with epilepsy. Bioavailable testosterone and ratings of sexual interest and sexual function were significantly lower in men with epilepsy treated with enzyme-inducing antiepileptic drugs compared with men with epilepsy taking non-enzyme-inducing antiepileptic drugs and men without epilepsy (Herzog et al., 2003). Notably, there were no differences in levels of bioavailable testosterone and the ratings of sexual interest and sexual function of men on non-enzyme-inducing antiepileptic drugs and men without epilepsy (Herzog et al., 2003). These data suggest that enzyme-inducing antiepileptic drugs may play a role in the altered endocrine and reproductive functions of men with epilepsy.

2.2. Altered gonadal function in response to hippocampally focused seizures

Recent data from our laboratory support the idea that seizures may alter androgens. Intact mid-aged rats that have had seizures have modestly altered androgen levels in the hippocampus compared with intact rats that did not have seizures (Table 4). Notably, there were no differences in the hippocampal androgen levels of GDX rats that did or did not have seizures (data not shown). These data suggest that an intact hypothalamic–pituitary–gonadal (HPG) axis may be necessary for gonadal responsiveness to seizures. This idea is consistent with reports from the clinical literature that men with epilepsy have premature and accelerated reductions in androgens in relation to age. Thus, it may be that men with epilepsy have a HPG axis that is less responsive at an earlier age or that ictal activity accelerates the aging process. As well, this may reflect the dose-dependent effects of androgens to modulate seizures. The administration of androgens to intact males that have endogenous androgens present may produce one effect, while administering androgens to males with very low endogenous levels may produce different effects. For these reasons, it is important to consider the effects of endocrine and developmental status when investigating androgens' effects on seizure processes.

In addition to androgen levels being altered by seizures, the expression of 5α-reductase, the enzyme necessary for testosterone's metabolism to dihydrotestosterone and 3α-diol, in the hippocampus is also altered by seizures. Using the 5α-reductase type 1 rabbit antirat antibody (kindly provided by Dr. David Russell, University of Texas, Southwestern Medical Center), we found that kainic acid administration significantly reduced the number of 5α-reductase type I immunopositive cells in the CA1 region of the hippocampus compared with vehicle administration (Fig. 9). Whether this effect is due to fewer cells or decreased 5α-reductase production is not known. However, it is clear that there are functional consequences of this change because androgen levels were altered, albeit modestly, in the hippocampus of rats that had seizures compared with those that did not. Indeed, in rats that had seizures, testosterone levels were higher compared with rats that did not have seizures. However, there was a negligible difference in dihydrotestosterone levels and a decrease in the 3α-

Table 4
Hippocampal T, DHT, and 3α-diol levels of intact mid-aged male rats that have or have not had seizures

Condition	Hippocampal androgen levels (ng/g)					
	T		DHT		3α-diol	
	No seizures	Seizures	No seizures	Seizures	No seizures	Seizures
Mid-aged	1.7 ± 0.4	2.6 ± 0.6	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.3	0.5 ± 0.3

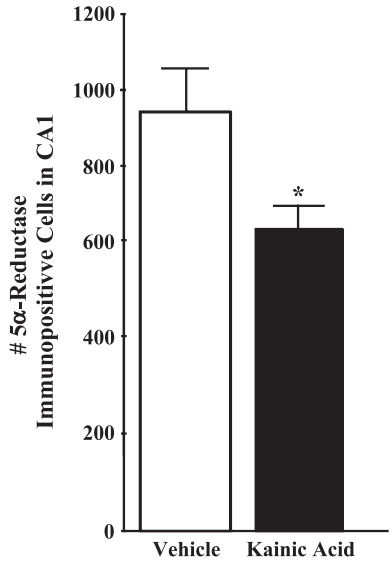

Fig. 9. Number of 5α-reductase type I immunopositive cells in CA1 of the hippocampus of rats administered vehicle (open bar, $n=8$) or kainic acid (32 mg/kg; black bar, $n=8$). * Significantly different from vehicle (Fishers Least Significant Difference Test, $P<.05$).

diol levels of rats that had seizures compared with those that did not. This pattern may be explained by a reduction in the 5α-reductase activity in the hippocampus. Testosterone is increased by seizure activity but is not as readily metabolized to dihydrotestosterone because of a decrease in 5α-reductase activity, which then leads to decreased metabolism to 3α-diol. Thus, while androgens may have profound effects on ictal activity, evidence suggests that this is a reciprocal relationship whereby seizures may also have the ability to alter androgen levels, which may then alter subsequent seizure processes.

3. Conclusions

The data from our laboratory presented in this review support androgens' antiseizure effects in the PTZ model and suggest that these effects may involve the actions of androgens in the hippocampus. Additionally, it appears that there may be a reciprocal effect of ictal activity on androgens. Ictal activity may not only be altered by androgens, but may also affect gonadal responsiveness. Although these data are important, there are many questions yet to be addressed.

First, the more recent data from our laboratory demonstrating that testosterone, dihydrotestosterone, and 3α-diol have similar effects to reduce seizure activity suggest that androgens' antiseizure effects may be mediated, at least in part, through the actions of 3α-diol. These data, together with previous data from our laboratory demonstrating that testosterone is not as effective at decreasing the ictal activity of mice lacking the 5α-reductase enzyme as in wildtype controls (Frye et al., 2001), support the idea that testosterone's 5α-reduced metabolites are important for antiseizure effects. Notably, 3α-diol can be back-converted to dihydrotestosterone. Thus, the effects of 3α-diol to decrease ictal activity could be due, in part, to the actions of dihydrotestosterone. Again, this lability in androgens may be related to the variable reports of androgens on seizure activity, and to date, we have utilized regimen that produce suprathreshold, rather than dose-dependent, effects of androgens to alter ictal activity.

Second, these data do not take into account testosterone's other metabolite, estrogen, which typically has proconvulsant effects in seizure models. Notably, the coadministration of testosterone and an aromatase inhibitor, testolactone, to men with epilepsy significantly decreases seizure frequency compared with testosterone alone (Herzog, 1999). As well, damage produced by seizures can up-regulate aromatase activity in the hippocampus (Garcia-Segura et al., 1999). Increased aromatase activity could alter the ratio of 5α-reduced to aromatized testosterone metabolites and, thereby, the effects of androgens on ictal activity. Thus, it is important to consider that androgens' antiseizure effects may depend upon the balance of testosterone's conversion to its 5α-reduced (dihydrotestosterone and 3α-diol) versus its aromatized (estradiol) metabolites, as well as androgen exposure relative to ictal activity (i.e., prior to, concurrent with, or after the initiation of seizure processes).

Third, stress can influence seizures (Haut et al., 2003), and both estradiol and 3α-diol are increased in response to stress (Erskine and Kornberg, 1992; Kuriyama and Shibasaki, 2004). These stress-induced elevations in hormones may also influence the ratio of 5α-reduced to aromatized metabolites and, thereby, subsequent seizure processes. Stress-induced increases in estradiol and/or 3α-diol may also produce dose-dependent effects to modulate seizures. For example, more stressful situations or environments may produce increases in central androgens, which may have additive effects with peripheral and/or exogenous androgens; thereby resulting in different effects on ictal activity than observed during less stressful situations. Most studies have failed to measure androgen and/or glucocorticoid levels to address the interactions between hormones, stress, and seizure processes.

Finally, although the evidence for androgens' actions in the hippocampus is compelling, it does not preclude actions of androgens in other brain areas that might be important for modulating seizure processes. Indeed, other brain areas have been implicated in seizure modulation, including the amygdala, periaqueductal grey, and pontine reticular formation (Feng and Faingold, 2002; Frye et al., 2000; Frye and Muscatiello, 2001; N'Gouemo and Faingold, 2000). Notably, androgens have proconvulsant effects in amygdala-kindled seizures, which suggests that androgens may exacerbate the effects of an already excited brain. Thus, it is clear that androgens' actions in other brain areas need to be investigated for their effects on seizures. Despite these remaining questions regarding androgens' modulation of ictal activity, the data reviewed and presented above

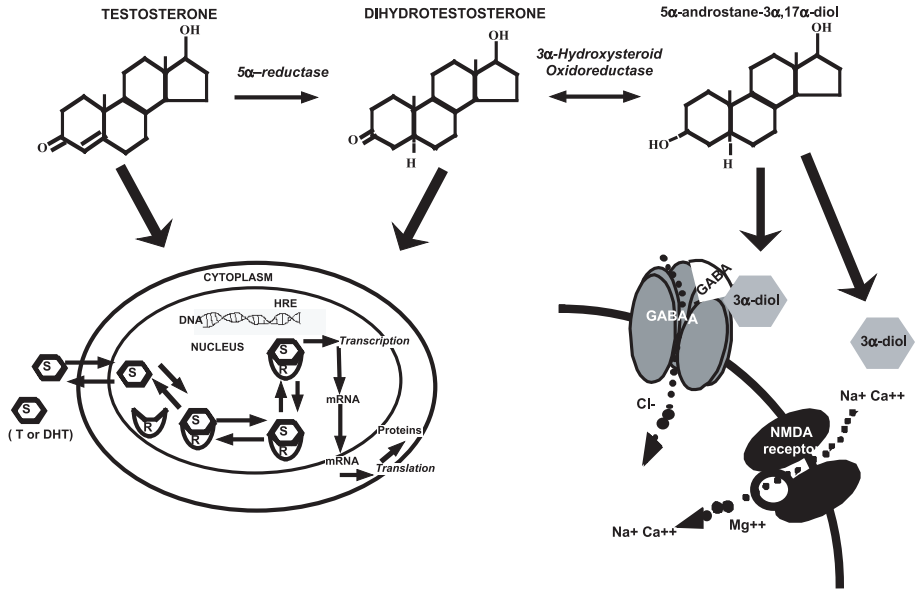

Fig. 10. Putative substrates of androgen's modulation of ictal activity.

suggest that this is an important area that merits continued investigation. Indeed, the data discussed above have been paramount in characterizing the effects of androgens on seizure processes.

3.1. Future areas of investigation

Although the antiseizure effects of 5α-reduced androgens that we have observed are important, they were produced by pharmacological regimen of androgens. Thus, an important question regards the threshold concentrations of androgens sufficient to decrease seizure activity. It is necessary to establish these levels to fully characterize androgens' effects on seizures and to begin to dissociate androgens' mechanisms for effects on ictal activity. Notably, testosterone and dihydrotestosterone have a high affinity for intracellular androgen receptors, while in physiological concentrations, 3α-diol does not (Cunningham et al., 1979). However, 3α-diol is a very potent modulator of $GABA_A$ receptors (Gee, 1988) and can alter the function of NMDA receptors (Pouliot et al., 1996). Thus, the extent to which androgens' antiseizure effects may be due, in part, to the actions of 3α-diol at intracellular androgen, $GABA_A$, and/or NMDA receptors (Fig. 10) will be investigated in the future.

To harness the full therapeutic effects of androgens for people with seizure disorder, further investigation of androgens' effects and mechanisms to modulate ictal activity is needed. Revealing the mechanisms of androgens' antiseizure effects may ultimately lead to enhanced therapeutic options for people with epilepsy. Data suggest that endogenous variations in androgens are associated with altered seizure susceptibility and that androgen replacement may ameliorate the effects of reduced or absent androgens. Thus, androgen replacement therapy, either alone or in conjunction with traditional antiepileptic drugs, may ultimately lead to the reduced use of traditional antiepileptic drugs and/or new antiepileptic drugs with fewer negative side effects.

Acknowledgements

This research was supported by grants from the Catherine Weldon Donaghue Foundation for Medical Research (96-001) and The National Science Foundation (IBN 95-14463; 98-96263; 03-16083; and 03-44103). The collaboration of Dr. Jacob Harney is appreciated. The technical assistance provided by Jonathon Madara, Joseph Madara, Neil Muscatiello, and Alicia Walf is also greatly appreciated.

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