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European Journal of Pharmacology 371 (1999) 147–152

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Ifenprodil and arcaine alter amygdala-kindling development [1]

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Received 8 March 1999; accepted 16 March 1999

Abstract

The NMDA receptor complex is thought to be altered in kindling, an animal model for complex partial epilepsy. This receptor complex has several modulatory sites including those for glutamate, glycine and polyamines with activation resulting in altered cation channel opening. Two NMDA receptor effectors, ifenprodil and arcaine, were evaluated for effects on the acquisition of electrical kindling of the amygdala. Rats were administered 0, 3.2, 10, 32 and 100 μg of ifenprodil or 0, 32 or 100 μg of arcaine, intracerebroventricularly, 10 min before a daily kindling stimulus. Ifenprodil, at low doses, enhanced kindling acquisition, while the highest dose, 100 μg, inhibited kindling. Arcaine increased the number of trials required to reach fully generalized (stage 5) seizures at the 100 μg dose. Since these agents had mixed actions on kindling development, it is unclear whether these or similar NMDA effectors would be useful in the modulation of complex partial seizures.

Keywords: Kindling; Seizure; NMDA receptor; Polyamine; Ifenprodil; Arcaine

1. Introduction

Kindling is recognized as an animal model for complex partial epilepsy, affording researchers the opportunity to study seizure maintenance as well as progressive development to include secondary generalization (McNamara et al., 1985). The kindling process involves periodically repeated, low intensity electrical stimulation of brain regions to induce acute after discharges which, with repetition, elicit progressively more severe electroencephalographic and motor responses (Racine, 1971; Goddard, 1983).

Excitatory amino acid receptors play a role in the genesis of kindling although the exact nature of this involvement is not completely clear. However, limbic seizures can be induced by repeated injections of glutamate, aspartate and *N*-methyl-D-aspartate (NMDA) into the rat amygdala and can be blocked by excitatory amino acid receptor antagonists shown also to be effective in electrical kindling (Croucher and Bradford, 1989; Croucher et al., 1995). Unilateral up-regulation of NMDA and non-NMDA glutamate receptors was found in the hippocampus and surrounding cortical brain regions of amygdala-kindled rat brain (Cincotta et al., 1991). Peterson et al. (1983) showed that the excitatory amino acid receptor antagonists, (±)-2-amino-7-phosphonoheptanoic acid (AP7), (±)-2-amino-4-phosphonobutyric acid (AP4) and (±)-2-amino-5-phosphonovaleric acid (AP5) blocked amygdala-kindled seizures. MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine), a noncompetitive antagonist (i.e., an open channel blocker) at the NMDA receptor complex, retarded kindling development and significantly suppressed seizure stage and after discharge duration of previously kindled amygdala seizures (McNamara et al., 1988; Morimoto et al., 1991).

Ransom and Stec (1988) found that spermine and spermidine, but not putrescine, increased [^{3}H]MK-801 binding to the NMDA ionophore with maximally activating concentrations of glutamate and glycine. From pharmacological and biochemical studies, it has become apparent that polyamines are modulators of the NMDA receptor complex (Reynolds, 1990a,b). During and after the amygdala kindling process, concentrations of putrescine but not sper-

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[1] Intramural research was funded by U.S. Army Medical Research and Material Command. Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 85-23. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4-3), AR 360-5.

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00183-1

midine and spermine were elevated (Hayashi et al., 1992). Putrescine administration was also shown to inhibit the development of kindling (Hayashi et al., 1992). While spermine and spermidine have been described as agonists (i.e., increasing binding of open channel blockers), putrescine blocks the effects of spermine and spermidine and, thus, has been described as an antagonist indicating, perhaps, that elevations in putrescine concentrations after kindling are compensatory to increases in excitatory neurotransmission.

Ifenprodil has also been identified as an antagonist at the polyamine site of the NMDA receptor complex (Schoemaker et al., 1990). It has little effect on seizures in fully kindled rats (Ebert et al., 1997), but has been shown to exhibit protective activity in focal ischemia (Gotti et al., 1988). Polyamines have been shown to both potentiate or inhibit NMDA ionophore activity depending on their concentrations, being that there are high-affinity stimulatory and low-affinity inhibitory binding sites for the polyamines (Ransom and Stec, 1988; Marvizón and Baudry, 1994; Johnson, 1996). Therefore, ifenprodil and other polyamine site antagonists could have diverse effects. Ifenprodil has been shown to be a weak open-channel blocker at NMDA receptors containing subunits NR1A/NR2A and to have competitive glycine site antagonist effects at receptors with NR1A/NR2B subunits (Williams, 1993).

Binding studies have also identified arcaine (1,4-diguanidinobutane) as a competitive antagonist at the polyamine site of the NMDA receptor (Reynolds, 1990a). Sacaan and Johnson (1990) also reported that arcaine produced a complete inhibition of [^{3}H]-*N*-(1-[thienyl]cyclohexyl)piperidine ([^{3}H]-TCP) binding or binding of an NMDA channel blocker) in rat cortical membranes. In addition, Donevan et al. (1992) reported that arcaine prevented MK-801 binding in the NMDA receptor channel although the arcaine binding site discussed was distinct from the channel dizocilpine or magnesium sites (Wang and Johnson, 1992).

Considering the role of the NMDA receptor in epilepsy as described above, it appeared that the mixed action NMDA receptor effectors ifenprodil and arcaine might well have potential for therapeutic manipulation in epileptic disorders. To expand our understanding of the polyamine site on the NMDA receptor complex in this type of epileptogenesis, ifenprodil or arcaine was administered during the development of kindling, a model of complex partial epilepsy.

2. Materials and methods

2.1. Surgical implantation of amygdaloid electrodes and a ventricular cannula

Male Sprague–Dawley rats (300–400 g) were anesthetized with an intraperitoneal injection of Chloropent (3 ml/kg; per ml: chloral hydrate (42.5 mg), magnesium sulfate (21.2 mg), pentobarbital (8.86 mg), ethyl alcohol (14.25%), propylene glycol (33.8%) or ketamine and xylazine (6 mg/kg xylazine and 70 mg/kg ketamine). At the time of deep anesthesia, the rat was placed in a stereotaxic frame, the scalp shaved and scrubbed with betadine and ethyl alcohol. The skull was exposed, the periosteum removed and the surface of the skull dried. Coordinates for both bregma and lambda were determined and the skull was levelled by adjusting the stereotaxic incisor bar such that there was no more than a 0.1 mm V difference between bregma and lambda. The skull was then marked and burr holes were drilled for passage of electrodes and positioning of screws. A depth electrode was placed in the basolateral amygdaloid nucleus (−2.2 mm AP, +4.7 mm LAT, −8.5 mm V, relative to bregma). A guide cannula (22 gauge, 8.5 mm in length; Plastics 1, Roanoke, VA) was positioned in the lateral ventricle using the following coordinates relative to bregma: −1.0 mm AP, −1.6 LAT, −4.5 V. Two screw electrodes were placed touching the surface of the frontal cortex and a retaining screw and a third screw electrode were inserted in the skull over the right and left parietal cortex, respectively. An acrylic mount was formed to fix the elements to the skull and provide for the construction of an external plug for monitoring of amygdala and cortical electrocephalography (EEG). A dummy cannula (28 gauge, 8.5 mm in length) was inserted in the guide cannula for maintenance of patency until the time of intracerebroventricular (i.c.v.) drug administration. The incision was closed around the acrylic mount and the surrounding area sprayed with Furazolidone.

2.2. Kindling protocol

Two weeks after surgery, with the use of an internal cannula (28 gauge, 9.0 mm in length such that there was a 0.5 mm projection beyond the tip of the guide cannula) inserted in the guide cannula, rats were administered (over 60 s) either 0, 3.2, 10, 32 or 100 μg of ifenprodil or 0, 32 or 100 μg of arcaine in 10 μl of sterile water 10 min before the daily kindling stimulus. Daily stimulations (1.0 s train of 1.0 ms biphasic pulses, 60 Hz, 200 μA base-to-peak; see Racine, 1971) were performed using a Grass S88 stimulator until five cumulative stage 5 seizures were elicited (using the rating scale of Racine, 1971): 1, immobility, mild facial clonus, twitching of vibrissae; 2, head-nodding, eye closure, increased severity of facial clonus; 3, unilateral forelimb clonus; 4, rearing and bilateral forelimb clonus; 5, rearing with loss of righting reflex, and generalized clonic seizure). Amygdala and cortical EEG were determined via the implanted bipolar amygdala and cortical screw electrodes connected to a Grass 79D polygraph with EEG amplifiers, model 7P511J. EEG was recorded for 15 min before and resumed directly after the interrup-

tion by the kindling stimulus. The number of stimulations required to obtain the first unilateral forelimb clonic seizure (stage 3), the first generalized clonic seizure (stage 5) and the fifth stage 5 seizure was used to evaluate antiepileptogenic properties of the agents being studied. The after discharge duration was also measured at each of these kindling events.

2.3. Histology

Upon completion of the kindling protocol, rats were euthanized with Euthanasia-6 solution (5 ml/kg of body weight), perfused with 10% formalin and the brains removed and immersed in 10% formalin. Serial 60 μM coronal sections were cut on a freezing microtome and stained with Cresylecht violet. Coronal sections were examined for histological changes and amygdala electrode and i.c.v. cannula placement. Only those rats which were found to have appropriate placement of electrode and cannula were included in the following statistical analyses.

2.4. Drugs

Ifenprodil tartrate was obtained from Synthelabo Recherche (L.E.R.S., Bagneux, France) or Research Biochemicals International (Natick, MA). Arcaine sulphate was purchased from Sigma (St. Louis, MO). Both compounds were dissolved in sterile water and injected i.c.v. as described above.

2.5. Data analysis

The dose–response data were evaluated by a one-way analysis of variance and an a priori test for multiple comparison of means (Least Significant Difference or LSD test) was used to compare individual treatment groups.

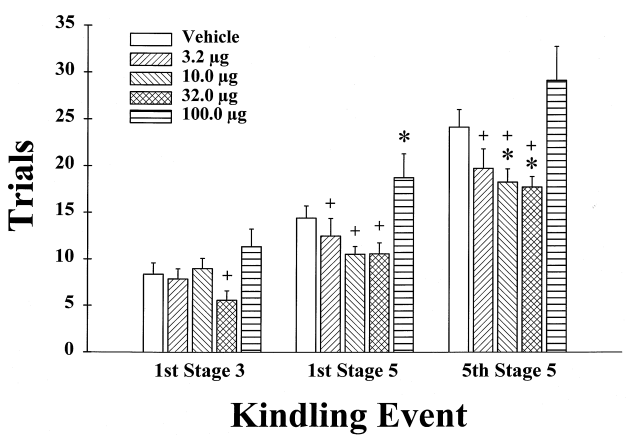

Fig. 1. The effect of ifenprodil on the number of trials to specific kindling stages. Bars represent the mean ± S.E.M. for vehicle ($n = 14$), 3.2 ($n = 5$), 10.0 ($n = 10$), 32.0 ($n = 11$) and 100.0 μg ($n = 10$) groups. $^{*}P < 0.05$ when compared to the vehicle group. $+ P < 0.05$ when compared to the 100.0 μg dose.

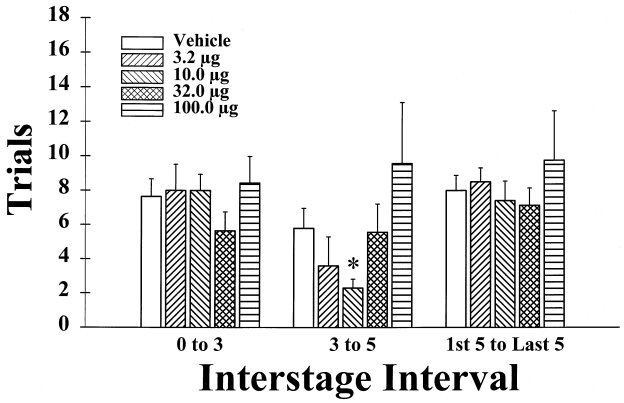

Fig. 2. The effect of ifenprodil on the number of trials between major kindling stages. Bars represent the mean ± S.E.M. for vehicle ($n = 14$), 3.2 ($n = 5$), 10.0 ($n = 10$), 32.0 ($n = 11$) and 100.0 μg ($n = 10$) groups. $^{*}P < 0.05$ when compared to the vehicle and 100.0 μg dose.

3. Results

Ifenprodil significantly reduced the number of trials (i.e., kindling enhancement) to the first stage 3 seizure, at the 32 μg dose, and to the first and fifth stage 5 seizure at 3.2, 10 and 32 μg doses. However, ifenprodil increased the number of trials (retardation of kindling) to the first stage 5 seizure at the high dose, 100 μg, when compared to vehicle controls (Fig. 1; $n = 8–21$) and the same tendency existed, although not statistically significant, at the fifth or last stage 5 seizure. In other words, lower doses enhanced kindling development while the high dose, 100 μg, significantly increased the number of trials (antagonism of the motor generalization of kindling development), albeit only to the first stage 5 seizure. The number of trials

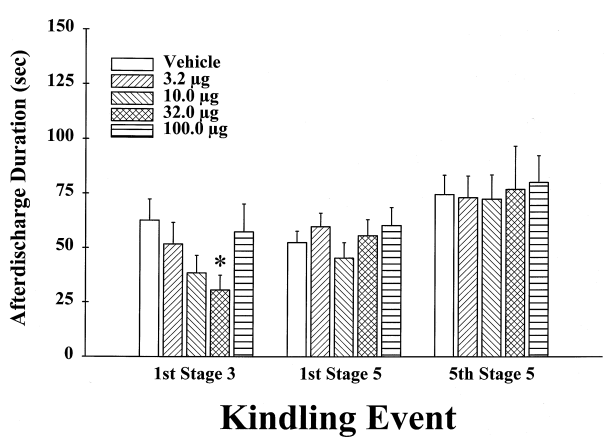

Fig. 3. The effect of ifenprodil on afterdischarge duration at specific kindling stages. Bars represent the mean ± S.E.M. for the vehicle ($n = 14$), 3.2 ($n = 5$), 10.0 ($n = 10$), 32.0 ($n = 11$) and 100.0 μg ($n = 10$) groups. $^{*}P < 0.05$ when compared to the vehicle group.

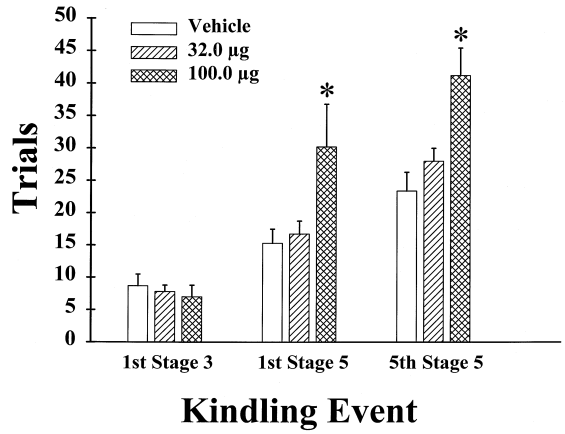

Fig. 4. The effect of arcaine on the number of trials to specific kindling stages. Bars represent the mean ± S.E.M. for vehicle ($n = 24$), 32.0 ($n = 6$) and 100.0 µg ($n = 8$) groups. $^*P < 0.05$ when compared to the vehicle group.

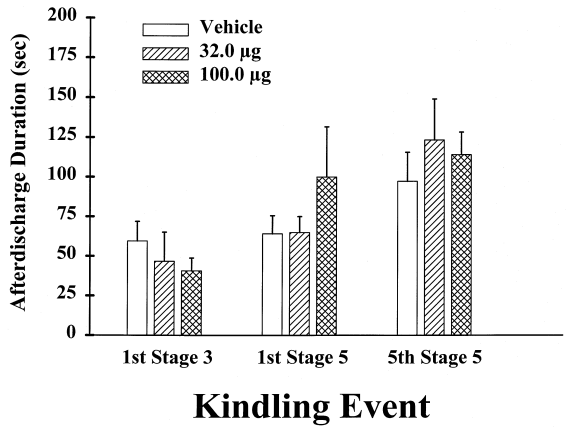

Fig. 6. The effect of arcaine on afterdischarge duration at specific kindling stages. Bars represent the mean ± S.E.M. for vehicle ($n = 24$), 32.0 ($n = 6$) and 100.0 µg ($n = 8$) groups.

between kindling milestones (interstage interval) indicated that, at least for the 10 µg group, statistically significant reductions in the number of trials occurred between stages 3 and 5 (Fig. 2). No other statistically significant differences were noted in interstage interval between ifenprodil and control groups because ifenprodil produced incremental changes in the number of trials between each kindling event. Ifenprodil had no pronounced effect on after discharge duration although a significantly shorter seizure duration at the first stage 3 seizure (Fig. 3) was seen with administration of 32 µg of ifenprodil.

Arcaine (100 µg) significantly increased the number of trials necessary to reach the first stage 5 and fifth stage 5 seizure milestone when compared to vehicle and the lower dose (32 µg; Fig. 4) which had no significant effect itself. This antagonism of kindling development occurred between the first stage 3 and the first stage 5 seizures as evidenced by the statistically significant increase in the number of trials required to reach the first generalized clonic seizure (stage 3 to 5: Fig. 5). Arcaine had no effect on after discharge duration at either dose tested (Fig. 6). Neither arcaine nor ifenprodil had any untoward effects on animal behavior.

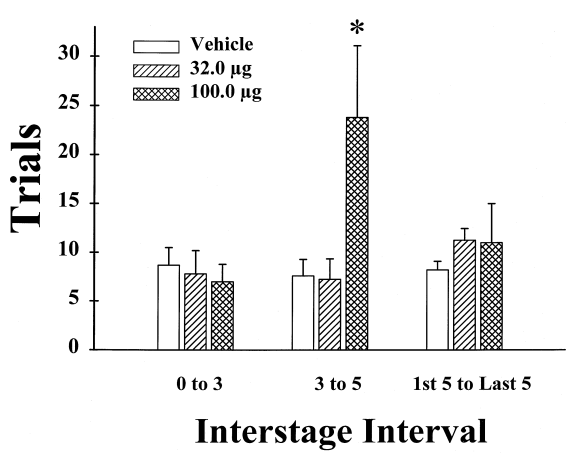

Fig. 5. The effect of arcaine on the number of trials between major kindling stages. Bars represent the mean ± S.E.M. for vehicle ($n = 24$), 32.0 ($n = 6$) and 100.0 µg ($n = 8$) groups. $^*P < 0.05$ when compared to the vehicle group.

4. Discussion

Ifenprodil wasfen found to be both a kindling enhancer at low doses (decreases number of stimulations to a kindling milestone) and a kindling antagonist at the high dose (100 µg; increases stimulation number), and to have little effect on after discharge duration. Ifenprodil has been shown to have a variety of actions in addition to those at the NMDA receptor complex that may explain diverse effects on kindling acquisition.

Arcaine (100 µg), like ifenprodil, significantly increased the number of trials necessary to reach the fully kindled or fifth stage 5 seizure (kindling antagonist) when compared to vehicle and the lower dose (32 µg). Arcaine had no effect on after discharge duration at either dose tested. Therefore, ifenprodil and arcaine appear to be antiepileptogenic, at least at high doses, but not anticonvulsant. Since ifenprodil and arcaine altered trials to each kindling event without an effect on after discharge duration, seizure spread was selectively manipulated while focal seizure activity was unchanged by drug treatment. However, unlike ifenprodil, the described effects of arcaine occur before fully generalized or stage 5 seizures since the interstage interval was only decreased from vehicle between the first stage 3 and the first stage 5 seizure.

It would seem that these observations are supportive of the view that the NMDA receptor is involved in kindling acquisition and that antagonists of the NMDA receptor can alter generalization of seizures (McNamara et al., 1988; Cincotta et al., 1991; Morimoto et al., 1991; Akiyama et al., 1992). However, it is difficult to say what pharmaco-

logical properties of ifenprodil are responsible for perturbations in kindling development. Ifenprodil is also a sigma ligand which has been described in the literature as a possible reason for antiischemic properties of this compound (Hashimoto et al., 1994). In addition, ifenprodil has α-adrenoceptor antagonist properties (Adeagbo and Magbagbeola, 1985) and is a vasodilator (Gotti et al., 1988). Gellman et al. (1987) showed that intraperitoneal administration of α_2-adrenoceptor antagonists enhanced kindling development while α_1-adrenoceptor antagonists had no effect. Ifenprodil is believed to be somewhat selective for antagonism of α_1-adrenoceptors suggesting that the adrenergic characteristics of ifenprodil probably do not explain the kindling effects noted in the current study based on previous research.

Ifenprodil blocks N- and P-type voltage-dependent calcium channels and was protective against ischemia-induced neurodegeneration (Church and Fletcher, 1995; Bath et al., 1996). It has been found that nifedipine and nimodipine, also calcium channel antagonists, are anticonvulsant or antiepileptic (Meyer et al., 1986; Yamada and Bilkey, 1991). Therefore, the kindling antagonist properties of ifenprodil could be due to blockade of voltage-gated calcium channels although arcaine has not been described as having this property and, like ifenprodil, was a kindling antagonist in the present study.

While the above studies would suggest that the mixed action of ifenprodil could be due to combined pharmacological effects, it may also be the result of the dual effect of the polyamines themselves on channel opening in the NMDA receptor complex. Specifically, polyamines are said to be able to inhibit or potentiate NMDA ionophore activity (Ransom and Stec, 1988; Williams, 1993; Marvizón and Baudry, 1994; Johnson, 1996) and thus, antagonists of these receptors may have diverse effects. However, since arcaine only antagonized kindling, this explanation is less probable but could be related to dosage of the individual agent administered. Indeed, putrescine, thought to be an antagonist of the polyamine recognition site of the NMDA receptor, increases the number of stimulations to full kindling acquisition (Hayashi et al., 1992) as did both arcaine and ifenprodil.

Ifenprodil has been shown to be a weak open-channel blocker at NMDA receptors containing subunits NR1A/NR2A and to competitively block glycine sites present on NMDA receptors with NR1A/NR2B subunits (Williams, 1993). Arcaine, while described as a competitive antagonist at the polyamine site of the NMDA receptor complex, can also depress NMDA binding when polyamines are not present and competitively prevents dizocilpine blockade of the NMDA receptor channel (Donevan et al., 1992). Indeed, the polyamines themselves can increase receptor affinity for glycine when glycine concentrations are low, potentiate channel opening at high or saturating glycine concentrations, and act as voltage-dependent blockers of the NMDA cation channel (Ransom and Deschenes, 1990; Sacaan and Johnson, 1990; Rock and MacDonald, 1992; Beneveniste and Mayer, 1993).

The present study supports previous findings that the NMDA receptor complex is involved in kindling acquisition. However, previous research has also shown that the effects of these compounds and the polyamines themselves are very complicated and, moreover, may have complex actions on certain populations of neurons or different neuronal circuits. Therefore, it is unclear whether compounds like ifenprodil and arcaine, which reverse or antagonize channel opening at the NMDA receptor complex, would be useful in the prophylaxis of complex partial epilepsy.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Shrividya Gopal, Nhut Tran and Rebecca Thorick.

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