The Effects of Atropine and Reserpine on Cortical Kindling in the Rat

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SUMMARY: *The effects on cortical kindling of atropine (a muscarinic, cholingergic blocking agent) and reserpine (a depleter of catecholamines and 5 hydroxytryptamine) were tested in this study.*

Atropine, which had previously been found to retard amygdaloid kindling, had similar but somewhat weaker effects on cortical kindling.

Reserpine also had similar effects on cortical kindling compared to subcortical kindling in that it potentiated seizure responses.

RÉSUMÉ: *Nous avons étudié l'effet sur le kindling cortical de l'atropine (un agent bloqueur cholinergo-muscarinique) et de la réserpine (un agent qui épuise les catécholamines et la 5-hydroxytryptamine.*

L'atropine, qu'on savait préalablement capable de retarder le kindling amygdaléen, a des effets semblables mais plus faibles sur le kindling cortical.

La réserpine a sur le kindling cortical des effets semblables à ceux sur le kindling sous-cortical en potentiant la réponse épileptique.

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The development of epileptiform responses provides an example of plasticity in mammalian neural systems. The kindling treatment is one of the more recent and perhaps the most easily controlled epilepsy model. This treatment involves spaced and repeated stimulation through implanted brain electrodes. The response evoked by low intensity stimulation progresses from no detectable response to a generalized electrographic and behavioral seizure (Goddard et al., 1969; Racine, 1972). So far, most of the forebrain areas tested have been found responsive.

Among the approaches used to investigate the underlying mechanisms of kindling are those that focus upon the role of the various neurotransmitter systems. Arnold et al. (1973), for example, found that a depletion of catecholamines increased the strength of seizure responses triggered by amygdala stimulation and increased the rate of amygdaloid kindling. In the same study it was reported that atropine, a cholinergic blocking agent, decreased the rate of kindling. The facilitation of amygdaloid kindling by catecholamine depletion was confirmed by Corcoran et al. (1974), and several laboratories have now reported depletions of noradrenaline and/or dopamine in the brains of kindled animals (Sato et al., 1975; Callaghan and Schwark, 1976; Engel and Sharpless, 1977). Corcoran et al. (1975) were not able to replicate the effects of atropine in Royal Victoria Hooded rats. Arnold (personal communication), however, has recently replicated the atropine effect in the Sprague-Dawley rat for the fourth time.

The effects of anticonvulsant drugs on the kindling phenomenon have also been tested (Babington and Wedeking, 1973; Racine et al., 1975). Racine et al., (1975) found a difference in the way the neocortex and the amygdala respond to phenytoin, procaine hydrochloride, and diazepam. Phenytoin and procaine were effective in blocking focal cortical responses, but they potentiated subcortical limbic responses. Diazepam was effective in blocking amygdala responses but had little or no effect on the focal cortical response at the same or double the dose level.

There are several possible mechanisms by which therapeutic drugs could be altering cortical and subcortical seizure activity. One possibility is that they are modifying the normal function of neurotransmitter systems (Pincus and Lee, 1973; Fuxe, et al., 1975). If these drugs are effecting seizure activity by altering the normal function of adrenergic or cholingeric transmitter systems, then it is possible that other drugs that effect these transmitter systems might also have different effects on cortical as compared to subcortical seizure responses. The experiments reported in this paper were designed to test that possibility by measuring the effects of atropine and reserpine on focal neocortical kindling.

METHOD

Twenty four male hooded rats, 250-300 gms, were used in this experiment. Bipolar .01 inch nichrome wire electrodes were implanted bilaterally into neocortical area 2 in all animals. The animals were then handled for 5 min a day for 5 days, beginning one week after surgery. Two weeks after surgery evoked potentials were measured.

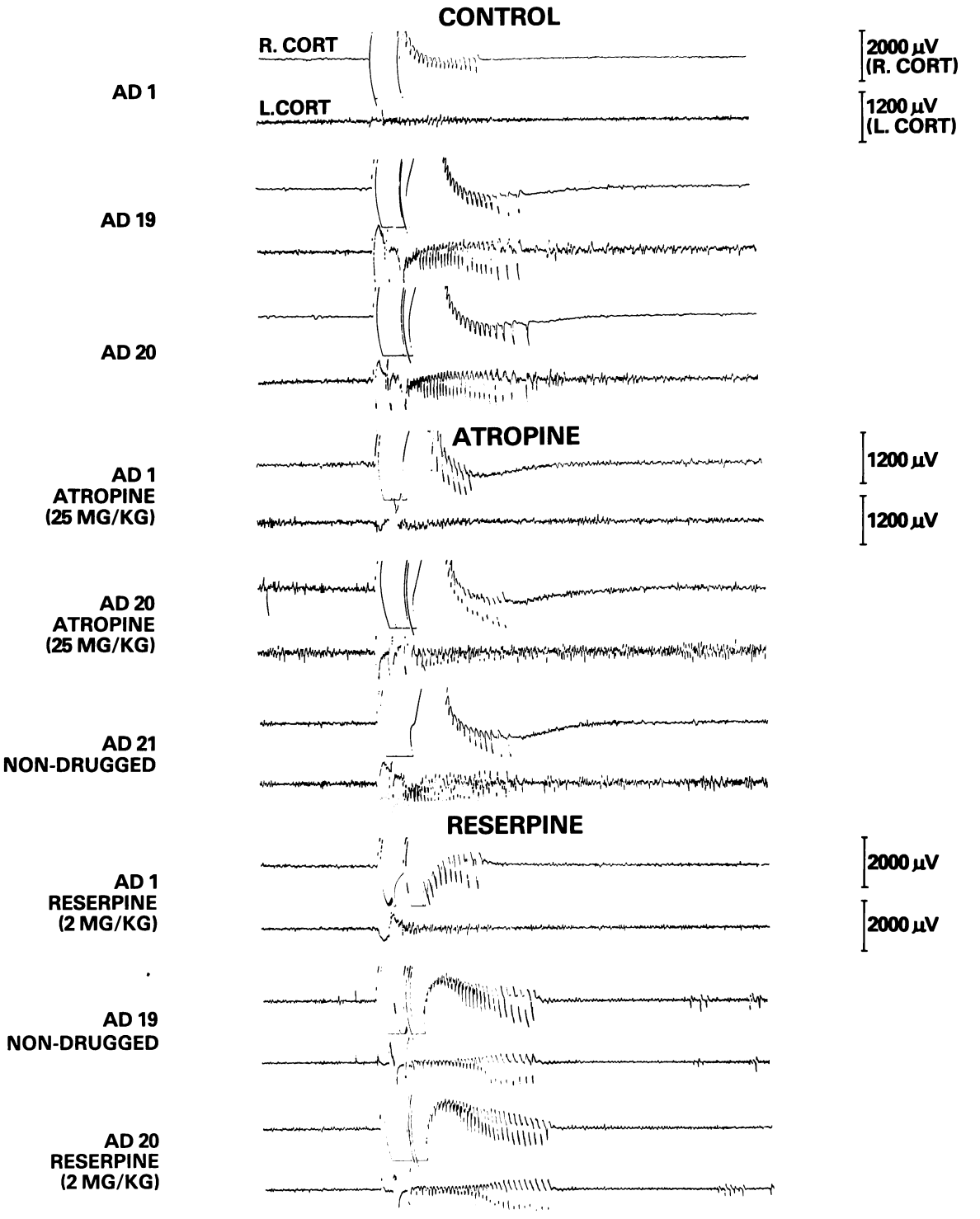

Figure 1—Sample after dishcharges from each of the groups tested in this study are shown above. The 1st, 19th and 20th discharges are shown for the control group in the top 3 rows. The first trace in each row is from the stimulated right cortex, and the second trace is from the contralateral homologous site. The 3 control discharges were all triggered in the non-drugged state.

The second 3 rows show the 1st (drugged) 20th (drugged) and 21st (first non-drugged) discharges from an animal in the atropine group. Note the slight increase in duration and the increase in propagation strength when the drug was removed (AD 21).

The final 3 rows show the 1st (drugged), 19th (non-drugged), and the 20th (drugged) discharges in the reserpine group. Note that reserpine appears to have little effect on either the 1st or last discharge when compared to the control traces or when compared to non-drugged traces in the same animal.

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Biphasic square wave pulses were applied through the right cortical electrode and the responses evoked in the left cortex were averaged. The pulse duration was 0.5 msec and the peak to peak amplitude was 400 μA. The average was based on a total of 50 responses. The EP responses were remeasured 4 days later as a test of stability. On the next day, 8 of the animals were injected IP with 25 mg/kg of atropine and the EP responses were remeasured 45 min later followed by the first kindling stimulation. Another 8 animals were injected IP with 2.0 mg/kg of reserpine and the EP responses were remeasured 24 hrs later followed by the first kindling stimulation. The 8 control animals were treated the same as the atropine animals except that they were injected with the same volume of physiological saline solution.

The kindling stimulation consisted of one sec of one msec biphasic square wave pulses at a frequency of 60 hz. The stimulation intensity was 400 μA, and the stimulation was applied to the right neocortex. The atropine was applied every other day one hr before the kindling stimulation until the animals had received 20 stimulations. The development of the electrographic discharge and the convulsive response was recorded and compared with the control animals. The reserpine treated group received the drug on only 3 occasions as previous work had indicated that animals deteriorate when given continual applications of reserpine. This group received reserpine 24 hrs prior to the first, tenth, and twentieth ADs.

At the end of the experiment, the brains were removed, fixed, sectioned and stained with thionin. The accuracy of the electrode placements was then checked using the light microscope.

RESULTS

Atropine and reserpine had no effects on cortical evoked potentials but they did have effects on cortical kindling that were similar to those previously reported for subcortical kindling (Arnold et al., 1973). Atropine retarded the development of the seizure response, particularly the convulsive response. The effect of atropine was relatively weak but significant. By day 20, the control animals had developed tonic components in the motor response, but the atropine treated animals had only developed a stronger forelimb clonus and were showing no signs of tonus. Figure 1 and Tables I and II summarize the drug effects on both the electrographic and the convulsive responses. Animals treated with atropine were developing seizure responses, but more slowly than the control subjects. When the atropine treated animals were taken off the drug and retested, the electrographic seizure responses were increased in duration and propagation strength (secondary site AD spike amplitude) (Table II). An additional 5-10 days of stimulation in the nondrugged state was sufficient to develop the seizure responses to the level of the control animals.

The reserpine treated animals showed drug induced increases in the strength of the convulsive but not the electrographic responses. In fact, there was a nearly significant tendency for AD spike amplitudes to be suppressed during reserpine treatments (Table II). Again, effects on the convulsions were not as strong as those previously found in animals receiving amygdaloid stimulation, but they were significant. Figure 1 shows the relative lack of effect of reserpine on the electrographic response at 2 different stages of cortical kindling. Reserpine appeared to increase the strength of the convulsive response at all 3 stages, but the effects at days 10 and 20 were much stronger than those at day 1 (Table I). During the nondrugged tests, before and after drug testing, the responses returned to levels nomal for that stage of kindling.

DISCUSSION

The fact that atropine and reserpine had similar effects on focal cortical as compared to amygdaloid kindling, and phenytoin, procaine hydrochloride and diazepam did not (Racine et al., 1975), suggests that the effects of those therapeutic drugs are not mediated via effects on cholingeric or catecholaminergic transmitter systems. It remains to be seen whether the effects of these drugs can be explained by their possible effects on other transmitter systems. It is possible that the effects of diazepam will be found to be a result of its effects upon GABA systems. Diazepam has been shown to activate GABA systems (Fuxe et al., 1975; Haefeley et al., 1975; Costa et al., 1975; Mao et al., 1975) and several types of experimental epileptiform response have been shown to be influenced by GABA levels. The effects of altering GABA levels on cortical and subcortical kindling are now being investigated in our laboratory.

The diphenylhydantoin and procaine effects may be due to the effects of these drugs upon calcium uptake across the membrane (Pincus and Lee, 1977). Both drugs block calcium uptake and we are currently investigating the effects of other drugs which block or facilitate the uptake of calcium.

Although atropine was found to retard cortical seizure development, the effect was weak. It is unlikely that cortical kindling effects could be explained by a facilitation of activity in cholinergic systems. The effects of atropine are probably due to a slight decrease in sensitivity in the neural systems that carry the seizure activity. Also, it is unlikely that kindling developments could be explained by a depletion or degeneration in catecholamine systems. Again, the effect is too weak. It is possible, of course, that the depletions must be more specific. There may be catecholamine systems with opposing functions so that a nonspecific depletion would have a weaker effect than a more specific depletion. It is also possible that kindling produces a nonspecific depletion but in portions of all or many of the inhibitory transmitter systems. Until we are forced to this hypothesis, however, our working assumption is that one or a few excitatory and/or inhibitory systems are primarily effected by the kindling treatment. Consequently, we, and others, are testing the effects of blocking agents on other transmitter systems as well as performing chemical assays on the brains of kindled animals.

TABLE I

CONVULSIVE RESPONSE

	Control			Reserpine			Atropine		
AD	1	10	20	1	10	20	1	10	20
Md	5.0	5.5	8.0	5.5	10.0	10.0	3.0	5.0	5.5
r	(1.0-5.5)	(1.5-9.0)	(5.5-10.0)	(1.5-10.0)	(5.5-10.0)	(9.5-10.0)	(1.0-5.5)	(1.0-7.5)	(3.0-9.0)

AD1: Overall (Kruskal-Wallis H-test):
H = 3.23, df = 2 NS

AD10: Overall:
H = 10.57, df = 2, $p < .01$

Pairwise (Mann-Whitney):
Control vs Reserpine U = 24, p = .08
Control vs Atropine U = 11.5, $p < .01$
Atropine vs Reserpine U = 4, $p < .001$

AD20: Overall:
H = 14.7, df = 2, $p < .01$

Pairwise:
Control vs Reserpine U = 17.5, $p < .05$
Control vs Atropine U = 15.5, $p < .02$
Atropine vs Reserpine U = 0, $p < .001$

TABLE II

Spike Amplitudes (μ Volts)

			Control: Primary Focus					
AD	1	2	9	10	11	19	20	21
X	655	766	850	866	896	788	791	843
r	(390-840)	(390-1250)	(450-1450)	(480-1450)	(390-1450)	(360-1200)	(450-1050)	(510-1290)
			Control: Secondary Focus					
AD	1	2	9	10	11	19	20	21
$\bar{X}$	305	269	545	564	584	609	725	654
r	(150-160)	(180-360)	(330-780)	(350-900)	(350-1000)	(240-1170)	(300-1410)	(270-950)
			Reserpine: Primary Focus					
AD	1*	2	9	10*	11	19	20*	21
$\bar{X}$	488	509	523	451	547	594	406	611
r	(90-1020)	(90-1020)	(160-1050)	(100-960)	(200-1110)	(300-1120)	(225-800)	(320-1050)
			Reserpine: Secondary Focus					
AD	1*	2	9	10*	11	19	20*	21
$\bar{X}$	473	528	841	723	832	1518	1091	1460
r	(200-1000)	(220-1800)	(360-2100)	(420-1100)	(420-1120)	(900-2250)	(550-1700)	(750-2150)
			Atropine: Primary Focus					
AD	1*	2*	9*	10*	11*	19*	20*	21
$\bar{X}$	608	776	1130	1139	1211	970	1041	1490
r	(350-950)	(400-1600)	(750-1550)	(700-1450)	(700-1650)	(890-1200)	(720-1400)	(1050-1950)
			Atropine: Secondary Focus					
AD	1*	2*	9*	10*	11*	19*	20*	21
$\bar{X}$	254	244	368	383	383	471	456	661
r	(150-450)	(150-360)	(210-480)	(270-510)	(210-540)	(240-810)	(210-810)	(150-1500)

*Days on which drugs were injected.

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