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Depletion of brain noradrenaline, but not dopamine, by intracerebral 6-hydroxydopamine potentiates convulsions induced by electroshock

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Intracerebral injection in rats of 4 μg of the catecholamine neurotoxin 6-hydroxydopamine was used to deplete forebrain noradrenaline to less than 10% of control values and separately to deplete brain dopamine to less than 15% of control. The susceptibility of these animals to electroconvulsive shock-induced convulsions was examined, and a significant potentiation of the response was seen in the rats depleted of noradrenaline but not in those depleted of dopamine. The duration of the convulsion was significantly increased as a result of loss of forebrain noradrenaline.

The catecholamines noradrenaline (NA) and dopamine (DA) have been implicated in the control of convulsions on a number of grounds. Thus, depletion of catecholamines by reserpine or α-methyl-*p*-tyrosine has repeatedly been found to potentiate the duration and severity of the convulsion induced by such agents as metrazol (leptazol), electroconvulsive shock (ECS) and audiogenic stimuli (Maynert 1969; Lovell 1971; Wenger et al 1973; Buterbaugh & London 1977). More recently the selective neurotoxin 6-hydroxydopamine (6-OHDA) has also yielded results indicating a role for catecholamines in convulsions (Corcoran et al 1973, 1974). However, the previously mentioned manipulations have often depleted both NA and DA simultaneously, making it difficult to determine which is of importance in the observed effects (Ayhan 1976; Lehmann 1977; Quattrone & Samanin 1977; Bourne et al 1977). A role of DA has been favoured on the basis of the findings that apomorphine will reduce the incidence of photically generated convulsions in Senegalese baboons (Meldrum et al 1975) and that the potentiation of convulsions found in response to the catecholamine-depleting agent Ro 4–1284 can be reversed by intracerebroventricular infusion of DA (Jobe et al 1974; Stull et al 1977). While a role for NA has been favoured by Mason & Corcoran (1978a,b) based on observations of a potentiation of metrazol-induced convulsions following depletion of forebrain NA.

We chose to examine the relative roles of NA and DA in the convulsions induced to response to electroconvulsive shock by depleting each amine separately using intracerebral injections of 6-OHDA.

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MATERIALS AND METHODS

NA depletion

Male albino Wistar rats (Woodlyn Farms, Ontario), 300 g at operation were anaesthetized with pentobarbitone (50 mg kg^{-1}, i.p.) and positioned in a stereotaxic apparatus. Two holes were drilled in the skull through which a 34 gauge cannula was lowered to the coordinates: AP + 2·6 mm from interaural line, ML ± 1·1 mm from midline suture at bregma, and DV + 3·7 mm from interaural line with the animal's head in the plane of König & Klippel (1963). This corresponds to the course of the ascending NA fibres in the dorsal bundle in the mesencephalon. 6-OHDA (4 μg) (expressed as free base of 6-OHDA HBr, Regis Chemicals) dissolved in 2 μl of 0·9% NaCl, containing 0·3 mg ml^{-1} ascorbic acid as antioxidant, were infused bilaterally at 1 μl min^{-1}, and the cannula was left in place for a further minute to permit diffusion of the drug. Control rats received infusion of saline-ascorbate. The skin was sutured and two weeks allowed for completion of anterograde terminal degeneration (Ross & Reis 1974).

DA depletion

Bilateral stereotaxic injections of 6-OHDA were performed as described above at the coordinates: AP + 5·9 mm from interaural line, ML ± 2·9 mm from midline suture at bregma, and DV ± 1·9 mm from interaural line. This corresponds to the course of the ascending DA fibres in the nigrostriatal

bundle. To prevent any loss of NA the animals were pretreated with the NA-uptake inhibitor (Evetts & Iversen 1970) desipramine (DMI, 25 mg kg^{-1} 30 min before operation). Since aphagia and adipsia leading to death can occur after this treatment the animals were tested beginning 72 h after the operation which is 24 h longer than Ranje & Ungerstedt (1977) have shown to be necessary for the depletion of striatal DA. To control for the possibility that a lesion-induced decline in food intake could alter susceptibility to convulsions, the control and lesioned rats were deprived of food for this period.

After completion of behavioural testing all animals were killed by cervical fracture, and then brains were rapidly dissected on ice into hypothalamus, hippocampus-cortex, and caudate. These regions were then assayed for endogenous catecholamines by the fluorometric method of McGeer & McGeer (1962). This served to confirm the extent and pattern of the amine depletion achieved by the two intracerebral 6-OHDA injection techniques.

Behavioural

Animals received transpinnae ECS through alligator clips wrapped in saline-soaked cotton wool attached to each ear. Three intensities of ECS were administered to the NA-depleted rats, each separated from the other by a two-day period. The lowest and the highest intensity were also administered to the DA-depleted rats. The intensities used were 10, 15 and 22·5 mA of 1 s duration, generated by an AC power source described previously (McCaughran et al 1974). The animal was then placed in a plastic cage; the clips were removed and the resulting convulsion was measured in terms of latency to onset, duration, and severity. The severity was assessed in terms of whether the animal showed clonic components only or whether it also showed tonic extension of the hindlimbs.

RESULTS

Biochemical

Table 1 shows the depletion of central catecholamines achieved by the injections of 6-OHDA into the dorsal bundle and into the nigrostriatal bundle. Following the dorsal bundle injection hippocampal-cortical NA was reduced to less than 10% of control concentrations and hypothalamic NA to 26%. Neither brain DA nor the NA systems projecting to cerebellum and spinal cord were significantly altered by this manipulation. Injection of 6-OHDA into the nigrostriatal bundle resulted in a reduction of caudate DA to less than 15% of control, with no significant alteration in NA in either hypothalamus or hippocampus-cortex. (Kelly et al 1975, have reported depletion of caudate DA to 49% of control values is effective in blocking amphetamine-induced stereotyped behaviour in rats.)

Table 1. Postmortem amine assays.

	Control (n = 10)	Lesioned (n = 10)	%
Dorsal bundle 6-OHDA			
Noradrenaline			
Hippocampus-cortex	264 ± 6	6 ± 1	2***
Hypothalamus	2240 ± 77	590 ± 87	26***
Cerebellum	219 ± 32	271 ± 35	124 NS
Spinal cord	255 ± 30	307 ± 22	120 NS
Dopamine			
Caudate	13100 ± 570	11600 ± 1190	88 NS
Nigrostriatal bundle 6-OHDA			
Noradrenaline			
Hippocampus-cortex	287 ± 28	263 ± 9	92 NS
Hypothalamus	1950 ± 115	1850 ± 85	95 NS
Dopamine			
Caudate	9820 ± 475	1390 ± 139	14***

Values are means with standard error of the mean in ng of amine g^{-1} wet weight of tissue. % column is the percentage of control concentrations remaining in lesioned tissues. The control and lesioned groups differed at the following levels of significance: ***, $P < 0{\cdot}001$; NS, not significant based on a two-tailed Student's t-test.

Behavioural

Table 2 shows the latency of onset, duration, and severity of ECS-induced convulsions in the NA and DA-depleted rats. No change in any parameter of the convulsion was seen following DA depletion.

Table 2. Electroconvulsive shock-induced convulsions.

	Control (n = 10)	Lesioned (n = 10)	P
Dorsal bundle 6-OHDA			
10 mA ECS	No. of animals showing tonic extension		
	4/10	2/10	NS
Duration of convulsion (s)	23·4 ± 4·3	35·4 ± 2·6	0·05
Latency to tonic extension (s)	3·5 ± 1·1	3·8 ± 0·9	NS
15 mA ECS			
	8/10	7/10	NS
Duration of convulsion (s)	24·3 ± 3·4	35·2 ± 2·3	0·01
Latency to tonic extension (s)	3·7 ± 0·5	3·6 ± 0·6	NS
22·5 mA ECS			
	9/10	8/10	NS
Duration of convulsion (s)	23·8 ± 3·2	27·8 ± 2·2	NS
Latency to tonic extension (s)	3·6 ± 0·6	2·6 ± 0·7	NS
Nigrostriatal bundle 6-OHDA	(n = 10)	(n = 10)	
10 mA ECS			
	4/9	3/10	
Duration of convulsion (s)	42·8 ± 5·1	47·7 ± 6·3	NS
Latency to tonic extension (s)	6·5 ± 0·5	7·3 ± 1·7	NS
22·5 mA ECS			
	9/9	6/10	NS
Duration of convulsion (s)	24·1 ± 2·0	26·3 ± 3·3	NS
Latency to tonic extension (s)	3·3 ± 0·8	2·5 ± 0·6	NS

Values are means with standard errors of the mean for NA-depleted (dorsal bundle 6-OHDA) and DA-depleted (nigrostriatal bundle 6-OHDA) rats.

However, following NA loss a marked potentiation of the convulsion was observed in terms of the duration of convulsion. Thus, at the 10 mA intensity of ECS the mean control duration was 23 s whereas that of the NA-depleted rats was 35 s (Mann–Whitney U test (Siegel 1956) = 20, $P<0{\cdot}05$). A similar effect was seen at the 15 mA intensity of ECS. The controls' mean duration was 24 s and that of the lesioned rats was 35 s ($U = 15$, $P<0{\cdot}01$). No change in the latency to onset was observed as a result of the NA depletion. The highest intensity of ECS studies (22·5 mA) was sufficient to overcome this potentiation by inducing maximal extension even in control rats (Table 2).

DISCUSSION

The present results confirm the involvement of catecholamines in the convulsive response to ECS and indicate a prime function of NA, rather than DA, in this effect (Mason & Corcoran, 1978a,b). The depletion of forebrain NA to less than 10% of control concentration resulted in a marked increase in the duration of the ECS-induced convulsion. Similarly, severe depletion of brain DA was without effect on this measure. This is in contradiction to findings indicating a role for DA in convulsions (Meldrum et al 1975; Jobe et al 1974; Stull et al 1977).

The NA lesion used here spared the NA projections to cerebellum and spinal cord. However, depletion of the spinal, but not the cerebellar, innervation has also been shown to potentiate the ECS-induced convulsion (Mason & Corcoran 1978c). Thus, it appears that NA has a pervasive role in the control and termination of convulsive activity. Whether this is a specific function for which the NA systems evolved or a more or less accidental consequence of their largely inhibitory synaptic action (Segal & Bloom 1974) is uncertain. In that a number of functions for the NA innervation of the forebrain have been demonstrated unrelated to convulsive activity (Mason & Iversen 1975, 1977a,b,c) the latter may be the actual case. Whatever the phylogenetic impetus for the development of the NA system in the forebrain it does appear to play a role in susceptibility to convulsions; and a disruption of this system in the clinical epilepsies may warrant further investigation.

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