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Brain areas involved in the catecholamine mediated regulation of electroshock seizure intensity

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Selective treatments which alter the catecholamine content of discrete areas of the brain were tested for their effect on electroshock seizure intensity in the rat. The data indicate that depletion of noradrenaline and dopamine in near ventricular areas by the intracerebroventricular administration of the benzoquinolizine, Ro 4-1284, enhances electroshock seizure intensity. The enhancement of seizure produced by systemic Ro 4-1284 was antagonized by the intracerebroventricular injection of noradrenaline or dopamine which do not appear to penetrate more than 1 mm into the brain. Further, pretreatment with systemic iproniazid and L-dopa completely antagonized the increased seizure intensity produced by intracerebroventricular Ro 4-1284 and repleted brain catecholamines in both near and far ventricular areas. Thus, the effects of both noradrenaline and dopamine in attenuating electroshock seizure intensity appear to be exerted principally through periventricular structures.

The role of noradrenaline and dopamine in the regulation of electroshock seizure in rats has been widely investigated (Prockop, Shore & Brodie, 1959; Rudzik & Johnson, 1970; McKenzie & Soroko, 1972; Kilian & Frey, 1973; Stull, Jobe & others, 1973; Jobe, Stull & Geiger, 1974; Jobe, Geiger & others, 1975). These studies support the concept that, in the rat, central nervous system noradrenaline and dopamine play an attenuating role in electroshock seizure. However, they do not define the specific area or areas of the central nervous system most directly involved in the modulation process.

The purpose of the present study was to determine the relative importance of near ventricular and far ventricular brain areas in the catecholamine-induced regulation of seizure intensity. Various procedures were employed to modify brain catecholamines and the effects of these treatments on seizure activity were observed. Since some of these treatments selectively modified the catecholamine content of discrete areas of the brain, it was possible to study the significance of these areas in the modulation of electroshock seizure activity. Relative biogenic amine content of specific areas was determined by a modification of the histochemical fluorescence technique of Falck & Owman (1965).

METHODS

Female Simonsen-derived Sprague Dawley rats (150–200 g) were used. All drugs were administered as solutions in normal saline except L-3,4-dihydroxyphenylalanine (L-dopa) which was injected intraperitoneally as a suspension in normal saline. Iproniazid phosphate was administered intraperitoneally while Ro 4-1284 (2-ethyl-1,2,3,4,6,7-hexahydro-2-hydroxy-3-isobutyl-9,10-dimethoxy-11bH-benzoquinolizine hydrochloride) was administered either subcutaneously or intracerebroventricularly. When injected intracerebroventricularly, Ro 4-1284, noradrenaline HCl and dopamine HCl were infused at a constant rate (10 μl min^{-1}) in volumes not exceeding 20 μl. Rats were prepared for intracerebroventricular injections by cannulae implantations according to Grunden & Linburn (1969).

The dosage regimens, test times and death times used are indicated in the Tables. In each set of experiments, test animals treated with a single drug were administered supplementary injections of saline and were handled and treated in the same manner as corresponding test animals given a combination of drugs. Controls were injected with saline and treated similarly to test animals.

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Electroshock procedure

Electroshock seizures were induced by stimulation of each animal with 26 mA (60 Hz sine wave stimulus) for 0·2 s through corneal electrodes. This procedure routinely produced clonic but not tonic seizures in all control (saline-treated) animals. The 26 mA stimulus used is well within the range (20–36 mA, average 28 ± 0·29 mA) reported for rats by Swinyard (1949).

The electroshock seizure pattern (either clonic or tonic) in rats was used to test the effects of selected drugs. Since the tonic pattern is more intense than the clonic pattern (Woodbury & Esplin, 1959; Swinyard, 1963), drugs which increase the incidence of tonus obviously enhance seizure intensity, whereas drugs which decrease the incidence of tonus, diminish seizure intensity. This method of assessing seizure intensity is routinely used in our laboratory (Stull & others, 1973; Jobe & others, 1974; 1975).

Histochemical fluorescence technique

The method used for determination of the relative catecholamine content of brain regions was a modification of that described by Falck & Owman (1965). Using this procedure, noradrenaline and dopamine content of brain areas rich in biogenic amines can be detected at concentrations as low as 15–20% of control (Kopin, Palkovits & others, 1974). Smaller amounts of biogenic amines cannot be visualized. However, according to Fuxe & Jonsson (1973), it appears safe to conclude that if a change in fluorescence intensity is visually detected, a true change in monoamine content has occurred.

The effects of selected treatments on near ventricular areas were determined by assessing the degree of catecholamine fluorescence in the anterior hypothalamic nucleus periventricularis, a structure located in juxtaposition to the 3rd ventricle. The effects on far ventricular areas were determined by assessing the degree of catecholamine fluorescence in the nucleus caudatus putamen at a site corresponding to 5780 μm anterior, 4200 μm lateral, and 400 μm dorsal as defined by the Konig & Klippel (1963) stereotaxic atlas. Amine concentrations were determined in these specific areas because of the high content of noradrenergic and dopaminergic nerve terminals.

Dissected portions of brain tissue were rapidly frozen in isopentane cooled with liquid nitrogen, lyophilized for three days at −30°, and then exposed for 1 h to formaldehyde gas generated from humidified paraformaldehyde at 80° (humidification was by exposure to an atmosphere of 58·3% relative humidity for at least five days before use). After exposure to formaldehyde gas, tissues were embedded in Paraplast (m.p. 56–57°) under vacuum and sectioned at 10 μm. Each section was mounted on a dry slide at 60–65° and viewed in a Leitz Dialux microscope equipped with a BG-12 excitation filter and a K-530 secondary filter. Photomicrographs were made with Kodak Tri-X Pan 35 mm film for visual comparison of relative degrees of fluorescence. Three animals were employed for each treatment procedure which involved histochemical fluorescence analysis.

Statistical procedure

The statistical significance of the difference between tonic seizure incidence values was determined by the Fisher exact probability test.

RESULTS

Effects of Ro 4-1284

Ro 4-1284 (a drug which has a brief reserpine-like action on biogenic amines) administered by the intracerebroventricular route significantly increased the intensity of electroshock seizure within 10 min of administration (Table 1). This enhancing effect on seizure was markedly diminished 60 min after injection. Histochemical fluorescence analyses, after treatment with Ro 4-1284, showed changes

Table 1. *Effect of intracerebroventricular Ro 4-1284 on electroshock seizure intensity and central neuronal catecholamine fluorescence in the rat.* I No. of rats with tonic extension (n = 8). II Fluorescent intensity compared with saline-treated rats.

Time after Ro 4-1284[a]	I	II NVA[b]
0 (control) i.c.v. saline	0	4 +
5	3	3 +
10	8 $P<0{\cdot}01$[c]	+
15	8 $P<0{\cdot}01$	+
30	8 $P<0{\cdot}01$	+
60	2	3 +
120	0	4 +

[a] Ro 4-1284 (400 μg/20 μl of saline) was injected into the right lateral ventricle at the rate of 20 μg μl^{-1} min^{-1} and the effects on seizure and amines were tested at the times indicated above.
[b] Near ventricular area: periventricular nucleus
[c] Significant difference from control.

in periventricular catecholamines which closely paralleled the effects on seizure intensity (Table 1). Decreased amine concentrations in near ventricular sites coincided with increased seizure intensity and as these sites were repleted seizure intensity returned to normal.

Effects of L-*dopa*

Systemically administered L-dopa, given in combination with iproniazid (a monoamine oxidase inhibitor), produced a dose- and time-dependent decrease in seizure intensity in animals pretreated with Ro 4-1284 (Tables 2 and 3). Concomitantly, a dose- and time-dependent increase in catecholamine fluorescence intensity was observed in parenchymal tissue in both periventricular and far ventricular areas.

Table 2. *Effect of* L-*dopa on Ro 4-1284-enhanced electroshock seizure intensity and central neuronal catecholamine fluorescence in the rat: an* L-*dopa dose-effect analysis.* I and II see Table 1.

L-Dopa (mg kg^{-1})[a]	I	II NVA[b]	II FVA[c]
0 (control)[d]	6	+	+
25	5	2 +	2 +
75	4	3 +	3 +
125	1	4 +	4 +
	$P<0{\cdot}050$[e]		
250	0	4 +	4 +
	$P<0{\cdot}01$		

[a] L-Dopa was administered intraperitoneally 1·5 h after iproniazid (40 mg kg^{-1}, i.p.) and 1 h after Ro 4-1284 (10 mg kg^{-1}, s.c.). Animals were tested 0·75 h after L-dopa.
[b] Near ventricular area: periventricular nucleus.
[c] Far ventricular area: nucleus caudatus putamen.
[d] Controls were treated with Ro 4-1284, iproniazid and saline (0·9%). All other animals received Ro 4-1284, iproniazid and L-dopa.
[e] Significant difference from control.

Table 3. *Effect of* L-*dopa on Ro 4-1284-enhanced electroshock seizure intensity and central neuronal catecholamine fluorescence in the rat: an* L-*dopa time-effect analysis.* I and II see Table 1.

Time after L-Dopa[a] (min)	I	II NVA	II FVA
0 (control)	7	+	+
5	6	2 +	2 +
7·5	4	3 +	3 +
10	0	4 +	4 +
	$P<0{\cdot}01$		

[a] L-Dopa (250 mg kg^{-1}, i.p.) was administered 1·5 h after iproniazid (40 mg kg^{-1}, i.p.) and 1 h after Ro 4-1284 (10 mg kg^{-1}, s.c.).
For other notes see Table 2.

Effects of noradrenaline

Intracerebroventricularly administered noradrenaline produced a dose-dependent antagonism of the Ro 4-1284-induced enhancement of electroshock seizure while also producing a dose-dependent increase in fluorescence intensity in brain areas close to the ventricle (Table 4). With regard to this latter point, fluorescence was observed only within 1 mm of the boundary of the ventricle even at the highest dose of noradrenaline administered. At distances greater than 1 mm from the ventricle, fluorescence intensity remained negligible at all doses of noradrenaline including those producing a distinct attenuating effect on seizure intensity.

Table 4. *Effect of intraventricularly administered noradrenaline and dopamine on the intensity of electroshock seizure and central neuronal catecholamine fluorescence in Ro 4-1284-treated animals: a dose-effect analysis.* I and II see Table 1.

	Dose[a] (μg)	I	II NVA	II FVA
Noradrenaline	0(control)[b]	6	+	+
	83	6	2+	+
	125	4	3+	+
	167	2	3+	+
	208	0	4+	+
		$P<0{\cdot}01$		
Dopamine	0(control)	8	+	+
	600	7	2+	+
	900	7	3+	+
	1200	2	4+	+
		$P<0{\cdot}01$		

[a] Noradrenaline and dopamine injections were made into the right lateral ventricle 85 min following Ro 4-1284 (10 mg kg^{-1}, s.c.) and 35 min before testing. Doses as the free base.
[b] Controls were treated with Ro 4-1284 and with intracerebroventricularly administered saline (0·9%) solution.
For other notes see Table 2.

Effects of dopamine

Intracerebroventricularly administered dopamine markedly reduced seizure severity in Ro 4-1284 pretreated rats and caused an increase in catecholamine fluorescence in near ventricular structures (Table 4). Areas further removed from the ventricles remained relatively low in catecholamine concentration at all dosage levels of dopamine.

DISCUSSION

Reserpine (which depletes storage sites) and Ro 4-1284 (which has a reserpine-like effect of short duration) increase electroshock seizure in rats (Chen, Ensor & Bohner, 1954; Stull & others, 1973). Furthermore, in these reserpine- or Ro 4-

1284-treated animals, repletion of brain catecholamines produced by administration of amine precursors or by central injections of noradrenaline or dopamine, completely antagonizes the increase in seizure intensity. For example, in animals with Ro 4-1284-induced depletion of catecholamine stores, partial repletion of noradrenaline and dopamine (by the administration of L-dopa plus iproniazid) antagonizes the increase in seizure intensity, but not in the presence of pimozide (a dopamine receptor blocking agent) administered in combination with diethyldithiocarbamate (a dopamine-β-hydroxylase inhibitor). This observation provides evidence for the effect of both noradrenaline and dopamine in the modulation of brain excitability.

The present study provides evidence that noradrenaline and dopamine within near ventricular sites are active in the modulation process. For example, administration of Ro 4-1284 into the ventricular system of rats produced an increase in seizure intensity and a simultaneous reduction in catecholamine fluorescence in brain parenchyma located in close proximity to the ventricular system.

Following subcutaneous treatment with Ro 4-1284, systemic administration of L-dopa and iproniazid produced a reduction of seizure severity and a concomitant repletion in catecholamine fluorescence within near and far ventricular areas. This observation does not allow the relative importance of specific central sites to be assessed.

In sharp contrast, intracerebroventricularly administered noradrenaline or dopamine failed to penetrate more than 1 mm into the brain parenchyma. Nevertheless, they completely antagonized the Ro 4-1284 increase in seizure severity. This observation implies that noradrenaline or dopamine can function as modulators of electroshock seizure within periventricular structures. However, in view of the inability of the histochemical fluorescence method to detect concentrations of catecholamines below 15–20% of control, small increases in brain areas somewhat further removed from the ventricles may also contribute to the seizure attenuating effects of intracerebroventricular noradrenaline and dopamine. Furthermore, although the direct effects of intracerebroventricular catecholamines may be predominantly within periventricular structures, the modulating effect of these monoamines on electroshock seizure may occur at more distant sites synaptically connected to catecholamine neurons of the periventricular structures.

Additional observations in this laboratory have shown that intracerebroventricularly administered 5-hydroxytryptamine (creatinine or oxalate salts) does not antagonize Ro 4-1284-enhancement of electroshock seizure, and in fact, produces generalized convulsions in higher doses. In view of previous findings by Prockop & others (1959) that systemically administered 5-HTP caused a distinct anticonvulsant effect in rats, our observations would indicate that structures other than periventricular areas are involved in the 5-HT mediated inhibitory effect.

In conclusion, data collected in the present study indicate that noradrenergic and dopaminergic nerve terminals located in near ventricular structures are functionally active in the attenuation of electroshock seizure. The possibility that far ventricular areas of the brain also participate in the attenuation process is not precluded.

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