EFFECT OF NEUROLEPTICS ON NORADRENALIN RESERVES IN PERIPHERAL NERVE FIBERS OF THE RAT

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The effect of chlorpromazine, trifluoperazine, and reserpine on the reserves of the adrenergic mediator in the rat vas deferens was studied by spectrofluorimetry, fluorescence histochemistry, and electron microscopy. Chlorpromazine (5 mg/kg) had no effect on the total content of noradrenalin, its distribution, and its granular reserves in the adrenergic nerves. Trifluoperazine (1 and 5 mg/kg) lowered the noradrenalin concentration in proportion to the number of granular vesicles (up to 70%), but by a lesser degree than reserpine (1 and 5 mg/kg).

An essential role in the mechanism of action of neuroleptics may be played by the decrease in the brain concentration of catecholamines produced by them [1, 4, 7, 9]. Because there is much in common between the mechanisms of function of the central and peripheral adrenergic neurons [2], it was decided to investigate the effect of trifluoperazine, chlorpromazine, and reserpine on the noradrenalin reserves in adrenergic nerve fibers of the rat vas deferens, a convenient model on which to study pharmacological effects on the content, intraneuronal distribution, and ultrastructural localization of the mediator.

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 180-250 g. Chlorpromazine in a dose of 5 mg/kg and trifluoperazine and reserpine in doses of 1 and 5 mg/kg were injected intraperitoneally. The animals were decapitated in the experiments with chlorpromazine and trifluoperazine after 2, 4, and 24 h and in the experiments with reserpine after 2, 4, 10, and 20 h. In each experiment the vas deferens was divided into three parts, in one of which the concentration of catecholamines was determined by the method of Von Euler and Lishajko [5], without attempting to differentiate between noradrenalin and adrenalin in the samples because of the low concentration of the latter. Fluorescence was recorded with the Opton spectrofluorimeter at wavelengths of 395 and 505 nm. Another part was treated by the fluorescence histochemical technique of Falck and Owman [6]. The sections were examined with the ML-2 luminescence microscope. The third part of the material was used for electron-microscopic investigation and cytochemical treatment by the method of Tranzer et al. [12], which permits the specific detection of monoamine reserves stored in the synaptic vesicles.

EXPERIMENTAL RESULTS

The results are summarized in Table 1. The mean concentration of noradrenalin in the control animals was 18.3 ± 1.4 μg/g. Its level did not differ significantly from the control 2, 4, and 24 h after administration of chlorpromazine. Trifluoperazine, in doses of 1 and 5 mg/kg, reduced its concentration by 24-36%. This effect continued at about the same level for the rest of the investigation. In the experiments with reserpine the noradrenalin concentration fell more appreciably than after trifluoperazine, and the effect was clearly dependent on the time of administration of the reserpine.

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TABLE 1. Concentration of Noradrenalin in Vas Deferens of Rat after Administration of Chlorpromazine, Trifluoperazine, and Reserpine

Substance	Dose (in mg/kg)	Time after injection (in h)	Noradrenalin concentration		Intensity of fluorescence	Number of granular vesicles (in % of control)
			in µg/g fresh tissue	in % of control		
Control			18,3±1,4*	100	++++	100
Chlorpromazine . .	5	2	16,4±2,4	90	++++	
		4	16,2±1,6	88	++++	93
		24	16,9±1,2	93	++++	96
Trifluoperazine . .	1	2	13,9±1,2	76	++++	
		4	13,1±1,7	71	++++	
		24	13,7±2,2	72	++++	
	5	2	12,1±1,8	66	++++	74
		4	13,1±1,5	71	++++	
		24	11,8±1,0	64	++++	72
Reserpine	1	2	11,0±2,3	60	++++	
		4	7,5±1,4	41	+++(+)	
		10	3,8±0,6	21	++	
		20	2,3±0,8	12	+	
	5	2	10,8±2,5	59	++++	
		4	7,3±1,0	40	+++(+)	45
		10	3,3±0,6	18	++	19
		20	2,0±0,8	11	+	12

*Confidence limits of mean for P = 0.05.
Legend: ++++ bright fluorescence; +++ moderate; ++ weak; + very weak.

Fluorescence-histochemical investigations carried out to demonstrate the mediator visually in situ and to determine its concentration, localization, and character of distribution showed that in the control animals noradrenalin was present almost entirely in the adrenergic nerves and gave the typical green fluorescence [13]. A dense plexus of terminal branches of axons containing brightly fluorescent varices along their length were demonstrated histochemically (Fig. 1A). After administration of chloropromazine and trifluoperazine the content and character of distribution of the monoamines did not differ appreciably from the control (Fig. 1B), and the intensity of fluorescence likewise was unchanged (Table 1). Reserpine induced a gradual decrease in the brightness of fluorescence of the monoamines. After 4 h the picture of fluorescence still resembled the control although individual varices gave a rather weaker fluorescence. By 10 h the fluorescence of most of the thin axons had disappeared, the varices were fewer in number, and they gave a weak fluorescence (Fig. 1C). After 20 h the fluorescence was almost completely extinguished except in solitary varices (Fig. 1D).

In the electron microscope the adrenergic fibers appeared as sections of round, oval, or elongated shape depending on their projection in the plane of the electron micrograph. In the sections from the control animals synaptic vesicles with a dense inclusion at the center were found (Fig. 2A); this consisted of the product of the cytochemical reaction of the monoamines stored in the vesicles [3, 10-12]. Besides granular vesicles, some terminals contained solitary agranular vesicles of the same size, having probably lost their monoamines [11, 12]. In some experiments the number of granular vesicles per square micron of section through the nerve fiber was calculated, using at least 20-30 sections from the same specimen for this purpose. The mean number of vesicles in the control experiments was 158 ± 29 (P = 0.05). The results of these experiments, expressed as percentages of the control, are also given in Table 1. After administration of chlorpromazine (5 mg/kg) the number of granular vesicles remained virtually unchanged. Trifluoperazine (5 mg/kg) reduced their number to 72-74% (Fig. 2B). Reserpine (5 mg/kg) had a stronger action: the number of granular vesicles 4 h after its administration was reduced to 45% (Fig. 2C), and the decrease continued down to 19% after 10 h and to 12% of the control level after 20 h (Fig. 2D).

The following conclusion can be drawn from a comparison of the results. Chlorpromazine, under the conditions investigated, had no effect on the total content of noradrenalin, its distribution in the presynaptic structures, and the state of its granular reserves. This agrees with the results of the biochemical determination of noradrenalin in the brain after administration of chlorpromazine [4]. Trifluoperazine reduced the total content of mediator to 64-76% and reduced the content of its granular reserves in proportion (down to 72%). The brightness of fluorescence and the localization and distribution of the monoamines in the adrenergic nerves were not visibly changed.

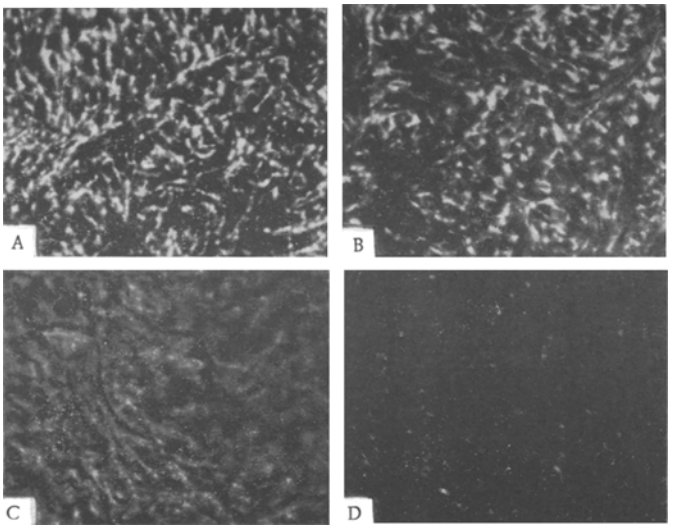

Fig. 1. Fluorescence of monoamines of adrenergic nerves of rat vas deferens after administration of trifluoperazine and reserpine (150×). A) Adrenergic fibers of control animal; B) 10 h after administration of trifluoperazine (5 mg/kg); C and D) 10 and 20 h, respectively, after injection of reserpine (5 mg/kg).

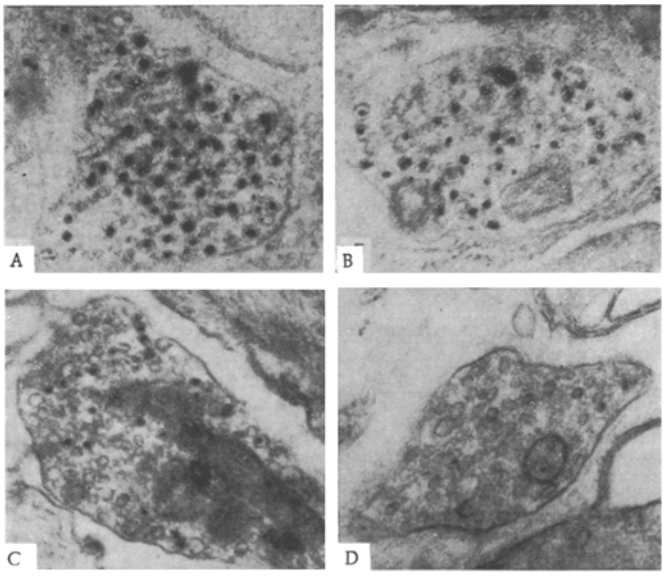

Fig. 2. Electron-microscopic demonstration of granular synaptic vesicles in adrenergic nerves of rat vas deferens after administration of trifluoperazine and reserpine: A) control; B) content of vesicles 4 h after injection of trifluoperazine (5 mg/kg); C and D) 4 and 20 h, respectively, after injection of reserpine (5 mg/kg). Magnifications: in A, B, D, 70,000×; in C 60,000×.

Reserpine exhausted the noradrenalin reserves most of all. Their total content, just as with trifluoperagine, fell in proportion to the decrease in granular reserves. Meanwhile the first 4 h, when the content of monoamines fell to 40%, showed virtually no change in the histochemical picture. Complete correlation between the histochemical findings and the results of the biochemical and electron-microscopic investigations was observed for the first time 10 and 20 h after administration of reserpine. The absence of such

correlation in the experiments with trifluoperazine and during the first few hours after administration of reserpine, when the noradrenalin content was reduced to 70 and 40%, respectively, of its initial level, agree with the observations of Jonsson [8] who showed that the relationship between the brightness of fluorescence of monoamine-formaldehyde derivatives and the noradrenalin content is linear only if the concentration of noradrenalin in the tissues does not exceed 40%. The decrease in the noradrenalin level in the endings of peripheral adrenergic neurons discovered by the writers in response to the action of trifluoperazine agrees with results of biochemical investigations of the brain [1].

The results described above are evidence of considerable differences between the actions of trifluoperazine, chloropromazine, and reserpine on the presynaptic structures of peripheral adrenergic neurons.

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