EFFECT OF NICOTINE ON NORADRENALIN CONTENT IN THE RAT BRAIN

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In doses of 0.4 and 4 mg/kg, nicotine reduced the noradrenalin (NA) content in the rat brain for 2 h. The greatest decrease in NA was observed in the period of hyperkinesia evoked by nicotine in a dose of 4 mg/kg. A further injection of the same dose of nicotine 30 min later did not give rise to hyperkinesia and did not induce any further decrease in NA. In a dose of 4 mg/kg given 4 and 20 h after reserpine (5 mg/kg) nicotine reduced the NA level and caused the appearance of hyperkinesia. Injection of nicotine (4 mg/kg) 25 min after amphetamine (5 mg/kg) did not significantly lower the NA level. Liberation of NA in the brain tissues is evidently an important central effect of nicotine and it shares a common mechanism with the effect of amphetamine.

KEY WORDS: rat brain; noradrenalin and its content; nicotine and its effect on the CNS.

The known effects of nicotine in the central nervous system (chiefly nicotine hyperkinesia) are explained predominantly by its central nicotinic (N) cholinomimetic action [1, 8, 11, 18]. Meanwhile the abolition of nicotine hyperkinesia is widely used as a test for the evaluation and screening of the central N-cholinolytics [5-7]. meanwhile, evidence continues to accumulate that nicotine is implicated in the metabolism, storage, and liberation of catecholamines in the peripheral tissues [19] and in the CNS [9].

The effect of nicotine, injected in different doses, including doses producing hyperkinesia, on the noradrenalin (NA) content in the albino rat brain was studied. The pharmacological analysis of the effects of nicotine was carried out with the aid of reserpine and amphetamine.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 170-200 g. The drugs were injected intraperitoneally. The development of hyperkinesia was recorded as the appearance or absence of tremor and clonic-tonic convulsions in the rats. For the determination of NA the brain was taken from the rats without the cerebellum and homogenized in 10 ml 5% TCA. NA was determined spectrofluorometrically by the method of Euler et al. [12, 13].

EXPERIMENTAL RESULTS AND DISCUSSION

Soon after injection of nicotine in a dose of 4 mg/kg all the rats developed hyperkinesia, which lasted for several minutes, after which the animals developed dyspnea and adynamia; after 2 h their condition was similar to what it had been previously. Nicotine in doses of 0.04 and 0.4 mg/kg did not produce externally visible changes in the rats' behavior. In half of the animals in each group of experiments the NA content in the brain was determined at different times after the injection of nicotine.

Biochemical analysis showed that nicotine, in a dose of 0.04 mg/kg, did not change the NA content in the brain in the course of 2 h after its administration. When nicotine was injected in a dose of 0.4 mg/kg the NA content fell after 30 min and returned to normal after 2 h. In rats receiving nicotine in a dose of 4 mg/kg a sharp decrease in the NA content was observed after 5 min and the NA level was significantly

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TABLE 1. Content (in μg/g tissue) of NA in Rat Brain at Different Times after Injection of Nicotine

Dose of nicotine (mg/kg)	Statistical index	NA content at different times after injection of nicotine							
		5 min		30 min		60 min		120 min	
		control	expts.	control	expts.	control	expts.	control	expts.
0,04	$M \pm m$	0,44±0,01	0,44±0,01	0,45±0,01	0,44±0,02	0,42±0,03	0,43±0,01	0,43±0,02	0,43±0,02
	% of control	100	100	100	97,7	100	102	100	100
	P				>0,7		>0,7		
	n	4	4	5	5	4	4	4	4
0,4	$M \pm m$	0,45±0,01	0,40±0,01	0,47±0,02	0,32±0,01	0,44±0,02	0,39±0,01	0,44±0,03	0,43±0,03
	% of control	100	89	100	68	100	88,6	100	97,7
	P		<0,001		<0,001		<0,005		>0,7
	n	6	6	10	10	4	4	4	4
4	$M \pm m$	0,43±0,01	0,25±0,02	0,44±0,01	0,34±0,01	0,44±0,02	0,39±0,02	0,44±0,02	0,43±0,01
	% of control	100	58,1	100	77	100	88,6	100	97,7
	P		<0,001		<0,001		>0,2		>0,7
	n	7	7	4	4	3	3	4	4

lowered for more than 30 min, i.e., actually during the period of hyperkinesia, after which it returned gradually to normal (Table 1).

Hyperkinesia induced by nicotine does not develop for 1-2 h after administration of a second dose [2]. This phenomenon was confirmed. It was shown that after hyperkinesia produced by the first injection of nicotine (4 mg/kg) a further injection of the same dose of nicotine did not cause hyperkinesia. In the rats of this group, no further decreases in the NA content was observed after a second injection of nicotine (Table 2).

In the experiments with reserpine (5 mg/kg) a considerable decrease in the NA level in the rats' brain was observed 4 h after its injection (0.12 ± 0.03 μg/g, or 28.6% of the control). The decrease in the NA level (0.22 ± 0.1 μg/g, or 52.4% of the control) continued 20 h after the injection of reserpine. Nicotine injected in a dose of 4 mg/kg 4 h after the reserpine induced hyperkinesia and lowered the NA reserves even more (0.06 ± 0.01 μg/g, 14.3% of the control). In experiments in which nicotine was injected 20 h after reserpine hyperkinesia also was observed, and in this case the NA level also was lowered (0.12 ± 0.01 μg/g, 28.6% of the control). The differences in the NA content between groups of animals receiving reserpine alone and reserpine with nicotine were significant ($P < 0.02$ after 4 h; $P < 0.001$ after 20 h).

Amphetamine in a dose of 5 mg/kg lowered the NA content in the brain after 30 min by 41% (0.26 ± 0.01 μg/g). Nicotine, injected in a dose of 4 mg/kg after amphetamine, caused a further decrease (0.20 ± 0.03 μg/g, 45.5% of the control) in the NA level, but the differences in the NA content in rats receiving amphetamine alone and rats receiving amphetamine and nicotine were not significant ($P > 0.05$). In the experiments in which nicotine was injected after amphetamine in a dose of 5 mg/kg, although hyperkinesia was observed its intensity was lower and its duration shorter. Accordingly, experiments were carried out on 16 rats which received amphetamine in a dose of 10 mg/kg, followed after 25 min by nicotine in a dose of 4 mg/kg. In 12 animals of this group nicotine hyperkinesia failed to develop completely and in 4 rats it was slight in degree. The brain NA level was not determined in this series of experiments.

The decrease in the NA content in the brain under the influence of nicotine was probably due to liberation of the mediator from the tissue depots. For example, the liberation of NA in the rat brain under the influence of nicotine was demonstrated by Bhagat et al. [10]. Similar results were obtained in experiments with perfusion of the guinea pig heart [20]. Hall and Turner [16] observed increased liberation of NA-H^3 from isolated sections of the hypothalamus when incubated with nicotine. The results of the present investigation (see also [3]) fully confirmed this view and showed that any attempt to explain the mechanism of action of nicotine must take into account its ability to induce the emission of the mediator from adrenergic nerve

TABLE 2. Effect of Repeated Injection of Nicotine on NA Content in Rat Brain

Treatment	No. of animals	Number of animals with signs of hyperkinesia		NA concentration (μg/g tissue)		
		after first injection of nicotine	after second injection of nicotine	$M \pm m$	P	% of control
Control	5	not injected		0,43±0,03	—	100
Nicotine, 4 mg/kg	5	5	not injected	0,24±0,01	<0,001	55,8
Nicotine 4 mg/kg + nicotine 4 mg/kg (30 min later)	5	5	0	0,23±0,03	<0,002	53,5

Note. In experiments in which a single injection of nicotine was given the NA content was determined 5 min after the injection; in experiments with two injections, it was determined 5 min after the second injection of nicotine.

endings both in peripheral organs and in the CNS. In this connection attention is drawn to the sharp decrease in the NA content arising in the brain actually during the period of nicotine hyperkinesia and to the absence of any further change in the NA level after a second injection of nicotine. These results, and also those of experiments in which nicotine was injected into rats after reserpine, demonstrate that an essential factor, perhaps the most important, in the mechanism of nicotine hyperkinesia, widely used as a test of excitation of central N-cholinergic receptors, is the intensive liberation of catecholamines in a physiologically active state. This hypothesis is confirmed by observations showing that nicotine convulsions in mice and rats are prevented by sympatholytics [17], and also by the complete prevention of nicotine hyperkinesia by the selective adrenoblocking agent pyrroxan [4].

Investigations by the present writers on reserpinized rats showed that, when the NA reserves were sharply depleted in the early stages after administration of reserpine, nicotine caused a further decrease in the level of the mediator. Meanwhile nicotine hyperkinesia also was observed. Hence it follows that nicotine causes the liberation of NA from reserves that have not been exhausted by the action of reserpine. The presence of such a reserve has been demonstrated by the study of the accumulation of biogenic amines in the CNS [14]. Meanwhile the experiments with consecutive injection of amphetamine and nicotine show that the action of these drugs in liberating NA evidently possesses a common mechanism. In both cases NA is liberated from nerve endings in a physiologically active state [15].

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