EFFECT OF SOME TROPANE DERIVATIVES ON NORADRENALIN UPTAKE BY SYNAPTIC VESICLES OF THE HYPOTHALAMUS

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The effect of some tropane derivatives on the uptake of exogenous noradrenalin was studied in experiments on isolated hypothalamic synaptic vesicles. LK-11, in a concentration of $1 \cdot 10^{-5}$ M, like cocaine, inhibits the passive uptake of noradrenalin. This effect was shown to depend on the concentration of mediator in the incubation medium.

KEY WORDS: tropane derivatives; hypothalamus; synaptic vesicles; noradrenalin.

Many neurotropic agents are known to inhibit the uptake of noradrenalin (NA) by the synaptic vesicles (SV) of nerve endings in the brain, so that the quantity of the physiologically active form of the mediator rises. In particular, isolated hypothalamic SV are capable of taking up exogenous NA [7, 8], and for that reason they can be used as an indicator of adrenergic mediation.

The effect of several tropane derivatives on NA uptake by the SV fraction of rat hypothalamus was investigated. The tropane derivatives studied included: tropinone hydrochloride (LK-1), tropine hydrochloride (LK-2), tropinone semicarbazide hydrochloride (LK-4), tropinone iodomethylate (LK-5), pseudotropine benzoate hydrochloride (LK-7), and the tropine ester of β-morpholinopropionic acid dihydrochloride (LK-11). Cocaine, whose central effects are connected with presynaptic blocking of the adrenergic mediator [3, 6], was used as the standard preparation.

EXPERIMENTAL METHOD

Groups of 10-12 rats were used. After decapitation the hypothalamic tissue was homogenized in 0.32 M sucrose solution. The unpurified mitochondrial fraction was isolated by the usual method at 12,000g (10 min), suspended, and subjected to osmotic shock by the addition of water to a sucrose concentration of 0.06 M. The SV fraction was isolated by the method of de Robertis et al. [2]. Heavy components (fraction M_1) were separated by the first centrifugation at 20,000g, after which the supernatant was centrifuged at 100,000g(for 1 h) to yield a residue consisting chiefly of SV (the M_2 fraction), and a supernatant containing soluble components (fraction M_3). To obtain the biochemical characteristics of the fractions obtained in these experiments, the distribution of NA and protein in them was studied and the relative specific concentration (RSC) of noradrenalin for each fraction was calculated.

SV were suspended in sucrose solution or Tris-HCl buffer, pH 7.4, so that 1 ml of suspension contained SV from 0.2 g tissue. Samples were then prepared, each of which contained 2 ml of suspension, 0.5 ml of NA solution (concentration given in the Tables), and 0.5 ml buffer, with or without addition of the test substances. The tropane derivatives and cocaine were used in a concentration of $1 \cdot 10^{-5}$ M. The samples were incubated for 15 min at 37°C or 0°C and centrifuged at 100,000g, after which the residual NA in the incubation medium was determined.

NA was estimated by a spectrofluorometric method [1] and protein by Lowry's method [4].

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TABLE 1. Distribution of Noradrenalin and Protein in Subcellular Fractions of Rat Hypothalamus (M ± m)

Subcellular fraction	Noradrenalin		Protein	RSC = % NA / % protein
	μg/g tissue	%		
M_1	0,80±0,09	40,0±1,7	62,4±3,9	0,64
M_2	0,73±0,08	36,3±1,7	19,5±2,5	1,86
M_3	0,47±0,06	23,7±1,8	18,1±2,1	1,31

Legend. Mean results of six experiments given

TABLE 2. Effect of LK-1, LK-2, LK-4, LK-5, and LK-7 on NA Uptake by Hypothalamic Synaptic Vesicles (M ± m)

Experiemental conditions	NA concentration	
	ng/ml	%
Control (without preparation)	50,0±7,6	100
LK-1	42,5±6,3	85
LK-2	47,5±8,8	95
LK-4	56,0±9,1	112
LK-5	50,0±9,0	100
LK-7	53,3±12,5	106
Cocaine	65,0±5,0	130*

Legend. Mean results of 3 or 4 experiments given. Asterisk marks values for which $P < 0.05$.

TABLE 3. NA Uptake by Hypothalamic Synaptic Vesicles in the Presence of LK-11 and Cocaine (M ± m)

Experimental conditions	Incubation	NA concentration (initial level 10^{-4} M)		NA concentration (initial level 3, 10^{-6} M)	
		ng/ml	%	ng/ml	%
Control (without preparation)	0°, 15 min	53,2±3,4	100	18,0±3,4	100
Control (without preparation)	37°, 15 min	38,7±2,6	72,7	20,0±4,5	111,1
LK-11	Ditto	44,7±3,5	84,0	19,5±3,5	108,3
Cocaine	"	46,4±2,7	87,2	23,0±4,5	128,0

Legend. Mean results of 4 experiments given.

EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 show that the fractions obtained by subcellular fractionation of the hypothalamic tissue were distinguished by a high NA concentration calculated per gram tissue, a characteristic feature of this part of the brain.

The highest RSC of noradrenalin (1.86) was obtained for fraction M_2, reflecting its high content of adrenergic SV. In the subsequent experiments this fraction was used as the model for studying the effect of tropane derivatives on NA uptake.

In the experiments of series I, SV were incubated in a medium of sucrose to which exogenous NA was added (2 μg/ml). Samples containing the test preparations or cocaine (10^{-5} M of each), and also the control (not containing the preparations), were incubated for 15 min at 37°C, after which they were centrifuged and the decrease in NA was determined in the incubation medium.

It will be clear from Table 2 that the tropane derivatives, in a concentration of 10^{-5} M, had no effect on NA uptake by the SV fraction. Under these same conditions cocaine blocked the uptake by 30% compared with the control.

Table 3 gives the results of the investigation of LK-11 which, as the writers have found, has a stronger pharmacological action than the other derivatives of this series. In the present experiments incubation was carried out in Tris-HCl buffer, pH 7.4, and in medium containing different NA concentrations ($1 \cdot 10^{-4}$ M and $3 \cdot 10^{-6}$ M). Clearly, with an NA concentration in the medium of $1 \cdot 10^{-4}$ M, its decrease after incubation (15 min, 37°C) was 27.3% compared with the control.

On the addition of LK-11 to the medium (10^{-5} M) moderate inhibition of NA uptake was observed. Under the same conditions cocaine had a similar effect.

In medium containing the smallest NA concentration ($3 \cdot 10^{-6}$ M) its uptake by SV was practically nil in these experiments. LK-11 and cocaine in this case also had no substantial effect on the NA concentration in the incubation medium.

Like cocaine, LK-11 thus inhibits the passive uptake of NA by hypothalamic synaptic vesicles in vitro. However, this effect depends on the NA concentration in the incubation medium. If the NA concentration is deficient, liberation of bound NA from SV can be activated [5]. This process also is characteristic of hypothalamic SV, but its sensitivity to pharmacological action may be different.

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EXPERIMENTAL STUDY OF INTERACTION BETWEEN GALANTHAMINE AND MOUSE BRAIN ACETYLCHOLINESTERASE in vivo

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Injection of armin* into mice after preliminary administration of galanthamine leads to a decrease in the inhibition of brain acetylcholinesterase (ACE) induced by the reversible inhibitor. This effect is associated with the accumulation of acetylcholine and displacement of galanthamine by it from the active centers of ACE. In experiments in vivo, the competitive character of interaction between galanthamine and ACE was thus revealed.

KEY WORDS: galanthamine and armin; brain acetylcholinesterase; reversible and irreversible inhibitors.

It was shown previously that galanthamine competes with acetylcholine (AC) for the active sites on acetylcholinesterase and that an increase in the substrate concentration in vitro leads to a decrease in the inhibition of ACE by this reversible inhibitor [1, 2].

*Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

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