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BRIEF COMMUNICATION

The Effect of Chronic Atropine Administration on Mouse Motility and on ACh Levels in the Central Nervous System

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MOLINENGO, L., A. FUNDARÒ AND M. ORSETTI. *The effect of chronic atropine administration on mouse motility and on ACh levels in the central nervous system.* PHARMACOL BIOCHEM BEHAV **32**(4) 1075–1077, 1989.—Changes in mouse motility and CNS cortical and subcortical ACh levels were studied after chronic (20 days) administration of 30, 40 and 60 mg/kg/day atropine. An increase in motility similar to that induced by acute atropine administration was observed, whereas the ACh levels reduction caused by acute administration was not repeated. These results suggest that changes in mouse motility caused by atropine are not correlated to its modification of ACh levels in the CNS.

Mouse motility ACh levels Atropine Chronic treatment

ATROPINE-induced depression of acetylcholine (ACh) levels in the CNS may be due to an antimuscarinic effect on the presynaptic ACh receptors (5). Raiteri *et al.* (7) have shown that these receptors undergo adaptive changes after chronic treatment with anticholinergic drugs. Inhibition of ACh release following their activation is thus increased. Therefore, it may be expected that the reduction of ACh levels observed after acute atropine administration is no more evident after chronic administration.

Moreover, if the increased motility caused by acute administration of atropine is correlated to an increased output of ACh and a reduction in ACh levels, tolerance of this increase would seem possible. To clarify these points, changes in mouse motility and CNS ACh levels were studied after chronic administration of atropine.

METHOD

Female albino Swiss Morini mice weighing 20–25 g were divided into groups of 20. Physiological solution (0.5 ml) was injected daily for 20 days in the control group. Atropine sulfate (30, 40 and 60 mg/kg/day) was injected subcutaneously for 20 days in the other 3 groups. These doses are approximately ⅛, ⅙ and ¼ of the acute LD_{50} of atropine in the mouse (6). Acute administration of 40 and 30 mg/kg atropine was also investigated in 15 animals. Fifteen mice were used as controls. A modification of the open field for rats (2) was used to evaluate spontaneous mouse motility. The floor of a square box (side 35 cm, peripheral wall 25 cm high) was divided into 25 squares (side 7 cm) and covered with a transparent plastic sheet to facilitate cleaning. A lamp (80-watt) at a distance of 150 cm provided uniform illumination of the floor. The mice were tested individually. To reduce or to eliminate the initial exploratory activity which may interfere with the evaluation of the simple motor activity, the mouse was put in the center of the box for ten minutes; in the subsequent 3-minute period the number of squares crossed was determined. A square was considered crossed only when the animal entered the square with all four paws. All experiments were performed in the morning at the end of the chronic pharmacological treatment.

At least 5 mice from each group were killed by microwave irradiation of the head on the same day of the test. The skull was opened and the brain frozen (−30°C). The brain was cut through the crus cerebri; the cerebellum and pons were discarded. The cortex and subcortex were collected and weighed. ACh was extracted by the method of Beani *et al.* (1). After homogenization in 2 ml of McIlvaine's citric acid disodium phosphate buffer, tissues were placed in boiling water for 30′, then transferred to ice-cold water and diluted with an equal volume of frog Ringer solution containing eserine salycilate (2.10^{-5} g/l) and a double salt concentration to obtain an isotonic medium. The extracts were centrifuged (3000 rpm) for 30 minutes. The supernatant was collected for the bioassay of ACh on the rectus abdominis of the frog according to the procedure given by the Staff of the

TABLE 1

EFFECT OF ACUTE ADMINISTRATION ON MOUSE MOTILITY AND CNS ACh LEVELS

		Squares Traversed in 3 Min	ACh Levels (μg/g fresh tissue) Cortex	Subcortex
Controls	mean ± SE	18.35 ± 4.35	2.92 ± 0.42	3.94 ± 0.53
	n	15	6	6
Atropine	mean ± SE	85.00 ± 21.33	1.51 ± 0.28	2.27 ± 0.31
30 mg/kg	n	15	6	6
	C.L.	−13.44 ÷ −119.96	+2.53 ÷ 0.29	+3.03 ÷ +0.31
Atropine	mean ± SE	94.00 ± 18.40	1.48 ± 0.25	2.07 ± 0.25
40 mg/kg	n	15	6	6
	C.L.	−22.64 ÷ 129.16	+2.56 ÷ 0.25	+3.23 ÷ 0.51

SE = standard error; n = number of animals; C.L. = confidence limits ($p = 95\%$) for the difference between each group mean and the control group mean (Dunnett's test).

Department of Pharmacology, Edinburgh (8).

The same procedures were used to evaluate motor activity (15 mice) and ACh levels (6 mice) after acute administration of 30 and 40 mg/kg atropine.

Dunnett's test was used to evaluate the significance of the differences between the controls and the treated mice.

RESULTS AND DISCUSSION

Acute administration of both 30 and 40 mg/kg atropine (Table 1) increased mouse motility and reduced cortex and subcortex ACh levels, as reported by other authors (3, 4, 9, 10). The number of squares crossed and cortex and subcortex ACh levels after chronic administration of 30, 40 and 60 mg/kg/die atropine are given in Table 2. It is clear that cortex and subcortex ACh levels are not modified by chronic atropine administration. Our results may be interpreted in the light of the observation (7) that the presynaptic muscarinic receptors which are inactivated by acute atropine administration undergo adaptive changes after chronic treatment with anticholinergic drugs.

Our data indicate that chronic administration of atropine causes significant increase of motility at doses which cause no modification of ACh levels. This discrepancy between the effects of chronic atropine treatment on ACh levels and motility suggest that motor activity is increased by a neurochemical effect of atropine differing from its effect on the presynaptic receptors.

TABLE 2

EFFECT OF CHRONIC ADMINISTRATION ON MOUSE MOTILITY AND CNS ACh LEVELS

		Squares Traversed in 3 Min	ACh Levels (μg/g fresh tissue) Cortex	Subcortex
Controls	mean ± SE	21.95 ± 2.58	2.53 ± 0.37	3.65 ± 0.31
	n	20	5	5
Atropine	mean ± SE	109.70 ± 3.98	2.15 ± 0.38	3.19 ± 0.51
30 mg/kg	n	20	6	6
	C.L.	−72.97 ÷ −103.3	+1.41 ÷ −0.65	+2.31 ÷ −1.39
Atropine	mean ± SE	164.50 ± 7.12	2.38 ± 0.14	4.04 ± 0.65
40 mg/kg	n	20	6	6
	C.L.	−127.52 ÷ −157.58	+1.18 ÷ −0.88	+1.46 ÷ −2.24
Atropine	mean ± SE	149.60 ± 2.33	2.83 ± 0.13	4.08 ± 0.41
60 mg/kg	n	20	5	5
	C.L.	−112.62 ÷ −142.68	+0.73 ÷ −1.33	+1.50 ÷ −2.7

SE = standard error; n = number of animals; C.L. = confidence limits ($p = 95\%$) for the difference between each group and the control group mean (Dunnett's test).

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