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BRIEF COMMUNICATION

# Action of Arecoline on the Levels of Acetylcholine, Norepinephrine and Dopamine in the Mouse Central Nervous System

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MOLINENGO, L., M. C. CASSONE AND M. ORSETTI. *Action of arecoline on the levels of acetylcholine, norepinephrine and dopamine in the mouse central nervous system.* PHARMACOL BIOCHEM BEHAV **24**(6) 1801–1803, 1986.— Modifications caused by arecoline (2 mg/kg and 10 mg/kg injected subcutaneously) in the levels of acetylcholine (ACh), norepinephrine (NE) and dopamine (DA) in the mouse cortex and "subcortex" were studied. The animals were killed by microwave irradiation of the head 15 minutes after drug administration. Arecoline 10 mg/kg caused a reduction in levels of ACh in the cortex and "subcortex" at the limit of statistical significance ($p$ 5–10%) and a statistically significant reduction in levels on NE. A statistically significant increase in DA was observed only in the cortex after 2 mg/kg and 10 mg/kg of arecoline.

Arecoline Acetylcholine Norepinephrine Dopamine Central nervous system Mouse

SEVERAL muscarinic agonists and acetylcholinesterase inhibitors increase brain concentration of acetylcholine [9, 12, 16, 18–20] and reduce the conversion of labelled precursors into Ach [5, 13, 19]. More recently it has been reported that the density of muscarinic receptors in cortical homogenates was significantly reduced in rats treated with diisopropylfluorophosphate (a well known acetylcholinesterase inhibitor) [4]. There is also ample evidence [2,21] of interactions between cholinergic and monoaminergic system in the brain. Therefore, we studied the modifications in the levels of acetylcholine (ACh), of norepinephrine (NE) and of dopamine (DA) in the mouse brain after treatment with arecoline, a cholinergic agonist, commonly used in behavioral studies.

## METHOD

Thirty-eight albino "Swiss Nos" mice (Nossan s.r.l., Correzzana/Mi) weighing 20–25 g were used. Arecoline hydrobromide (Serva, Heidelberg), 2 mg/kg and 10 mg/kg, was injected subcutaneously, dissolved in saline solution.

The dose of 2 mg/kg of arecoline is in the range of doses which, given subcutaneously or intraperitoneally, depressed self stimulation [10], spontaneous motor activity and operant behaviour in the rat [11]. Arecoline 10 mg/kg is a dose which may be considered at the limit of acute toxicity ($LD_{50}$70 mg/kg).

There is ample evidence that the behavioural modifications caused by arecoline began at 5–8 min from the administration and lasted 15–20 min [8, 10, 11] and therefore we killed the animals 15 min after drug administration.

The control mice (n=15) received 1 ml of saline isotonic solution subcutaneously.

The mice were killed 15 minutes after drug administration by microwave irradiation of the head (1.5 sec). The skull was opened and the brain frozen (−30°C). The brain was cut through the crus cerebri; cerebellum and pons were discarded. The cortex was collected and weighed. The remaining part of the brain ("subcortex") was also weighed.

Acetylcholine was extracted by the method given by Beani *et al.* [1]. The tissue, after homogenization in 2 ml of McIlvaine's citric acid disodium phosphate buffer (0.014 M, pH 4), was kept for 60 sec in boiling water, then transferred to ice cold water and diluted with an equal volume of frog Ringer solution containing eserine salycilate ($2\times10^{-5}$ g/l) and a double salt concentration to obtain an isotonic medium. The extracts were centrifuged (3000 rpm) for 30 min. The supernatant was collected for the bioassay of ACh on the rectus abdominis of the frog. The precedure given in [17] was followed. The contractions of the rectus abdominis caused

TABLE 1

EFFECT OF ARECOLINE ADMINISTRATION ON ACETYLCHOLINE, NOREPINEPHRINE AND DOPAMINE LEVELS IN THE MOUSE CENTRAL NERVOUS SYSTEM

| | | Acetylcholine | | Norepinephrine | | Dopamine | |
|---|---|---|---|---|---|---|---|
| | | cortex | "subcortex" | cortex | "subcortex" | cortex | "subcortex" |
| Controls | means ± S.E.M.* | 21.66 ± 2.30 | 29.92 ± 1.98 | 0.75 ± 0.11 | 0.77 ± 0.13 | 1.56 ± 0.26 | 2.52 ± 0.37 |
| | N† | (9) | (9) | (6) | (6) | (6) | (6) |
| Arecoline 2 mg/kg | means ± S.E.M.* | 21.75 ± 4.01 | 32.89 ± 0.55 | 0.73 ± 0.13 | 0.69 ± 0.08 | 4.16 ± 0.73 | 3.52 ± 0.56 |
| | N† | (6) | (6) | (6) | (6) | (6) | (6) |
| | *t*‡ | 0.0890 | 0.8592 | 0.0775 | 0.5237 | 3.0760 | 1.5376 |
| | *p*§ | >90% | 40–50% | >90% | 60–70% | 1–2% | 10–20% |
| Arecoline 10 mg/kg | means ± S.E.M.* | 15.18 ± 2.42 | 20.90 ± 4.18 | 0.37 ± 0.12 | 0.34 ± 0.11 | 3.13 ± 0.33 | 2.83 ± 0.80 |
| | N† | (6) | (6) | (5) | (5) | (5) | (5) |
| | *t*‡ | 1.8362 | 2.0481 | 2.3131 | 2.4547 | 3.6645 | 0.4044 |
| | *p*§ | 5–10% | 5–10% | 2–5% | 2–5% | 0.1–1% | 60–70% |

*Data expressed as nmoles/g fresh tissue.
†Number of animals.
‡Student's *t*-test for the differences between controls and treated animals.
§Probability of a causal result.

by the extracts were abolished by d-tubocurarine ($3\times10^{-6}$ g/l) and the contractions were also abolished when the samples were treated, before the introduction of eserine, with 0.7 unit/5 ml of bovine acetylcholinesterase (Sigma, Heidelberg) for 30 min at 37°C.

For the preparations of the sample of cortex and "subcortex" utilized in the evaluation of the levels of norepinephrine (NE) and of dopamine (DA), the same procedure was used. The samples were homogenized in perchloric acid solution and the method given by Shellenberger *et al.* [14] was followed. The NE fluorescence (activation peak 380 nm, fluorescence at 495 nm) and the DA fluorescence (activation peak 325 nm, fluorescence at 380 nm) was read in a Turner Mod 430 spectrophotofluorometer.

## RESULTS AND DISCUSSION

The levels of ACh, NE and DA found in the cortex and in the "subcortex" of the controls and of the mice treated with arecoline 2 mg/kg and 10 mg/kg are given in nmoles/g of wet tissue in Table 1.

The statistical significance of the differences between controls and treated mice was evaluated with the Student's *t*-test for the differences between means, and the probability of a chance result is given in the table. The results indicate that there is a reduction of the levels of ACh and of NE in the cortex and in the "subcortex" only after 10 mg/kg of arecoline and that there is an increase in the level of DA at 2 mg/kg and at 10 mg/kg of arecoline only in the cortex. We observed that the reduction of ACh levels in cortex and "subcortex" is not in agreement with the report of Haubrich *et al.* [6] in the whole rat brain. Species differences (rats vs. mice) might account for these discrepancies. In this context, it must be noted that the doses of arecoline (50 mg/kg) used by these authors are in the range of the lethal dose ($LD_{50}$ 70 mg/kg) and the observed effect might be an aspect of the acute toxicity of arecoline. It may also be noted that Haubrich *et al.* [6] killed their rats by cervical fracture and postmortem events may have produced a conspicuous modification of ACh levels. In fact, in controls Haubrich *et al.* [6] found about 10 nmoles/g of ACh in the whole brain of the rat. Our results indicate that in the mouse the control levels of ACh were 22 nmoles/g of ACh in the cortex and 30 nmoles/g in the "subcortex." Our data are in agreement with those of other authors. For example, in rats killed with microwave irradiation Cohen *et al.* [3] found about 26 nmoles/g and Hirsch *et al.* [7] found 36.5 or 38.5 nmoles/g in the whole brain of the rat. In any case the modifications we observed at a rather high dose of arecoline (10 mg/kg) are at the limit of statistical significance ($p$ 5–10%) and one may reasonably raise serious doubts that these neurochemical alterations are correlated with behavioural modifications.

A reduction of catecholamines was observed at 8 hours [15] from the death of the animals. In our experiments the extraction of catecholamines began within 10 minutes after the death of the animals. In control animals, the levels of NE found in the cortex (0.75±0.11 nmoles/g) and "subcortex" (0.77±0.13 nmoles/g) and the levels of DA found in the cortex (1.56±0.26 nmoles/g) and "subcortex" (2.52±0.37 nmoles/g) were similar to those reported by Sloviter and Connon [15] in the whole brain of the rat (NE: 0.78±0.06 nmoles/g and DA: 2.5±0.15 nmoles/g). The NE levels (Table 1) are reduced only at rather high doses (10 mg/kg) of arecoline and their statistical significance is low ($p$ 2–5%). The increase in DA levels observed at low doses of arecoline with a good statistical significance suggests a possible relationship between the increase in DA levels and the behavioral actions of arecoline. The changes in neurotransmitter level after administration of a muscarinic cholinergic agonist may be secondary to stimulation of muscarinic receptors that are present on dopaminergic or other types of neurons; there is ample evidence [2,21] of changes in DA levels and in DA release after administration of muscarinic agonists.

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