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An analysis of the catecholamine content of the salivary gland of the cockroach

J.P. FRY*, C.R. HOUSE & D.F. SHARMAN

Department of Physiology, Royal (Dick) School of Veterinary Studies, University of Edinburgh & Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

There is evidence that dopamine might be a neurotransmitter in the salivary gland of the cockroach *Nauphoetea cinerea* Olivier. Electrical stimulation of the salivary nerve results in hyperpolarization of the gland cell membrane (House, 1973). This effect is mimicked by adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine (5-HT). The effect of the catecholamines or nerve stimulation, but not that of 5-HT, is antagonized by phentolamine. The gland, however, does not respond to some other α- or β-adrenoceptor stimulating drugs (House, Ginsborg & Silinsky, 1973). Microspectro-fluorimetry has indicated the presence of a catecholamine in the nerve terminals in the gland (Bland, House, Ginsborg & Laszlo, 1973). Using a radiochemical assay for the simultaneous measurement of adrenaline, noradrenaline and dopamine we have investigated the catecholamine content of the cockroach salivary gland. The method is a development of that described by Cuello, Hiley & Iversen (1973). Twelve salivary glands, dissected as described by House (1973), were homogenized in 20 μl 0.1 M ice-cold perchloric acid, 10 μl saturated KCl solution added and the mixture centrifuged. 10 μl of the supernatant was incubated for 15 min at 37°C with 25 μl of a mixture, slightly modified from that described by Cuello *et al.* (1973) containing [^{3}H]-methyl S-adenosyl methionine and catechol-0-methyl transferase (prepared from pig liver). After the incubation, 25 μl of a solution containing metanephrine, normetanephrine and 3-methoxytyramine (10 mg/ml) was added followed by 10 μl 1 M perchloric acid and 20 μl saturated KCl solution. The mixture was extracted three times with ethyl acetate (250 μl) to reduce reagent blank radioactivity. A sample (50 μl) of the aqueous phase was treated twice with 10 μl acetic anhydride and solid $NaHCO_3$ to acetylate the methoxy derivatives of the catecholamines; these were extracted into ethyl acetate and separated by paper chromatography (see Sharman, 1971). The appropriate regions of the chromatogram were visualized by spraying with conc. ammonia solution, then with Folin and Ciocalteau's solution and finally with conc. ammonia solution. The chromatogram was cut into consecutive 1 cm portions and each portion placed in 10 ml Unisolve scintillation fluid for measuring radioactivity.

Three radioactive regions, not present on reagent blank chromatograms, were observed on chromatograms derived from salivary glands. The first did not coincide with any of the three radioactive regions derived from the catecholamines. The second lay beneath the region corresponding with acetyl normetanephrine but the Rf of the peak of the radioactivity (0.299 ± 0.009 (s.e. mean) $n = 8$) did not coincide ($P < 0.01$) with that (0.260 ± 0.008 (s.e. mean) $n = 8$) derived from added noradrenaline. The third region (Rf 0.745 ± 0.007 (s.e. mean) $n = 8$) coincided with that derived from added dopamine (Rf 0.737 ± 0.004 (s.e. mean) $n = 8$).

The salivary gland of the cockroach contains 0.55 ± 0.07 ng dopamine/gland (mean ± s.e.; $n = 8$) but the evidence does not establish the presence of noradrenaline.

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The effect of Δ^8-tetrahydrocannabinol (Δ^8-THC) on dopamine metabolism in the rat corpus striatum: the influence of environment

J.M. LITTLETON & K.I. MACLEAN*
(introduced by G. BROWNLEE)
Department of Pharmacology, King's College, London

Many workers have tried to relate behavioural actions of THC and brain monoamines with varying success. In some of its actions (e.g., catalepsy, hypothermia) THC closely resembles the neuroleptics which appear to block dopamine (DA) receptors as well as altering central noradrenaline (NA) metabolism (see Andén, Corrodi & Fuxe, 1972). It is therefore of interest to examine the effects of THC on central monoamine, and particularly dopamine, metabolism. In these experiments, depletion of rat brain catecholamines 2 h after administration of α-methyl p-tyrosine methyl ester (α-MT) (400 mg/kg i.p.) was used as an index of catecholamine turnover. Pooled (4) hypothalami or corpora striata, dissected as described by Glowinski and Iversen (1966), were analysed fluorimetrically for monoamines (NA, DA + 5HT). Male, Wistar rats (120-150 g) were kept in groups of six at 21°C and fed and watered freely before each experiment.

Δ^8-THC (10 mg/kg i.p.) administration under the above environmental conditions produced no change in regional brain monoamine concentrations, and did not alter the depletion of catecholamines when administered simultaneously with α-MT. Similarly, Δ^8-THC administration caused no change in monoamine concentrations in brains of rats subjected to isolation (24 h), food deprivation (24 h), or acute cold stress (2 h at 4°C). Under these conditions, however, the α-MT-induced depletion of DA in the corpus striatum was reduced by simultaneous administration of THC, whereas NA depletion in corpus striatum and hypothalamus was unaffected. The vehicle for THC (Tween 80, 4% in saline) did not affect catecholamine concentrations or the depletion produced by α-MT. Thus, it appears that striatal DA depletion after inhibition of tyrosine hydroxylase is inhibited by THC in animals subjected to isolation plus food deprivation, and isolation plus acute cold stress. Our results suggest that food deprivation is the most effective environmental factor studied in revealing this effect of THC.

These experiments suggest that, under certain environmental conditions, Δ^8-THC reduces striatal DA turnover without affecting other monoamines. It is not clear why we obtained these changes only under these environmental conditions. Food deprivation, isolation and cold stress may all change DA metabolism *per se* which may be relevant, but possible effects on drug absorption, distribution and metabolism cannot be discounted.

In conclusion, we suggest that reduction in central DA turnover in the corpus striatum by THC may explain some of its actions (e.g., hypothermia and catalepsy) and an asymmetrical reduction could explain the turning behaviour described by Waters & Glick (1973). Thus, although THC shares some properties with the neuroleptics, these could be mediated by inhibition of DA release rather than by DA receptor antagonism.

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