Effect of muscimol on dopamine metabolism of the rat hypothalamus[1]

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Summary. Muscimol, a potent GABA receptor agonist, produced increases in DOPAC and dopamine concentrations in the rat hypothalamus. GABAergic receptors, therefore, modulate hypothalamic dopaminergic neurones.

The hypothalamus is known to contain GABAergic neurones and receptors[2-4]. Muscimol (3-hydroxy-5-aminomethyl-isoxazole), a conformationally restricted GABA analogue, is a potent agonist at bicuculline-sensitive GABA receptors[5] and increases dopamine (DA) turnover in the corpus striatum and olfactory tubercle[6,7] after systemic and intracerebral administration. Since the hypothalamus contains dopaminergic neurones of the tubero-infundibular and incerto-hypothalamic pathways[8], the aim of this study was to examine the effect of muscimol on dopamine metabolism in the hypothalamus.

Materials and methods. Male Sprague-Dawley rats (150–250 g) were used in all experiments. Muscimol hydrobromide[9] was dissolved in 0.9% saline and injected either i.v. into the tail vein (1 ml/kg) or i.p. (2 ml/kg). Control animals received the appropriate volume of saline. Animals were sacrificed at various times after drug treatment, their brains rapidly removed and chilled in ice, and the hypothalamus (including the mammillary body) dissected[10](mean weight 51 ± 1 (50) mg). Tissue was homogenized in 20 vol. of ice-cold 0.1 M aqueous hydrochloric acid containing 0.1% EDTA and centrifuged at 10,000 × g for 10 min at 4 °C. Portions of the supernatants were assayed for DA, 3,4-dihydroxyphenylacetic acid (DOPAC), noradrenaline (NA) and 3,4-dihydroxyphenylethyleneglycol (DOPEG) by radioenzymatic methods employing catechol-O-methyltransferase (COMT, E.C. 2.1.1.6) and ^{3}H-methyl-S-adenosyl-methionine[11-15].

Briefly, homogenate supernatants (50 µl) were incubated with freshly prepared incubation mix (70 µl) which contained partially purified COMT (265 µg protein), 0.86 M Tris buffer pH 9.1, 12.7 mM $MgCl_2$ and 2 µCi of ^{3}H-SAM for 25 min at 37 °C. The tubes were placed in ice, 0.5 M boric acid pH 10 containing carriers (30 µl) was added, and each sample vortexed. Toluene/3-methylbutan-1-ol (500 µl, 3:2) was added, the samples vortexed and the organic phase isolated. ^{3}H-Methoxyamines were back extracted from the organic phase into 25 µl of 0.1 M aqueous hydrochloric acid. Portions (15 µl) were chromatographed on Whatman No. 1 paper to isolate ^{3}H-3-methoxytyramine and ^{3}H-normetanephrine[11]. The organic phase was evaporated and the residue reconstituted in 25 µl of 0.1 M Tris buffer pH 9.0 containing 0.1% EDTA. Portions (15 µl) were analyzed by paper chromatography[12] to separate ^{3}H-3-methoxy-4-hydroxyphenylethyleneglycol. The initial aqueous phase was retained, acidified with 0.4 M aqueous perchloric acid (900 µl), and ^{3}H-homovanillic acid was isolated by chromatography on Sephadex G-10 and solvent extraction[13,14]. In all cases the radioactivity present as ^{3}H-methoxyderivatives was estimated by liquid scintillation spectrometry, and quantitated using internal standards added to tissue extracts and external standards[15]. These methods allowed the determination of 30 pg of DA, 115 pg of DOPAC, 50 pg of NA and 35 pg of DOPEG.

Results. The effects of muscimol on the concentrations of catechols in the rat hypothalamus are summarized in the table. All schedules of muscimol elevated hypothalamic DOPAC, although the increases were not significant following a dose of 2 mg/kg administered i.p. The concentration of DA in the hypothalamus was significantly elevated by both 5 and 10 mg/kg i.p., whereas lower doses of muscimol failed to alter DA concentrations. Muscimol produced differential effects on hypothalamic NA with a decrease seen after i.v. administration and an increase when the drug was given i.p. Control levels of DOPEG were marginally below the sensitivity of the assay. However, the hypothalamic DOPEG concentration was measureable following muscimol at a dose of 2 mg/kg i.v., and a 40%, but insignificant, increase was seen relative to an estimated control level of 30 ± 5 (5) ng/g wet wt of tissue. Behavioural effects[16,17] were observed at all doses of muscimol.

Discussion. Our experimental data show that i.v. or i.p. injected muscimol can alter DA metabolism in the hypothalamus of the rat. The increase in the concentration of DOPAC in the hypothalamus correlates well with the increase observed by other workers in the corpus striatum and olfactory tubercle[6]. The elevation in hypothalamic DA levels at high doses of muscimol is also consistent with reports of increased striatal DA levels at similar doses[17]. DOPAC, the major acid metabolite of DA, has been used as a biochemical index of dopaminergic nerve activity and the concentration of DOPAC increases with increased firing of nigrostriatal and mesolimbic neurones[18]. By analogy, the rise in DOPAC concentration in the hypothalamus could be the result of increased activity of hypothalamic dopaminergic neurones.

Dopaminergic neurones of the tubero-infundibular pathway are known to inhibit prolactin secretion and elevated plasma prolactin levels are associated with increased activity of these neurones[8]. Muscimol has been reported to inhibit prolactin secretion[19]. The observed elevations in hypothalamic DOPAC after muscimol could be associated with the ability of this isoxazole to act at GABAergic

Effect of muscimol on catechols in the hypothalamus of the rat

Dose (route)	2 mg/kg (i.v.)		2 mg/kg (i.p.)		5 mg/kg (i.p.)	10 mg/kg (i.p.)
Time (min)	30	60	30	60	60	60
DOPAC	163 ± 8[d]	124 ± 13	109 ± 15	130 ± 21	165 ± 22[d]	174 ± 32[d]
DA	100 ± 20	101 ± 13	117 ± 11	109 ± 5	137 ± 7[c]	133 ± 13[c]
NA	81 ± 14[a]	80 ± 16	120 ± 6[b]	106 ± 12	94 ± 6	121 ± 13[b]

All values are the mean ± SE of 3 or 4 determinations and are expressed as percentage of control. Control values obtained from 16 animals were DOPAC 136 ± 12, DA 297 ± 39 and NA 892 ± 57 ng/g wet weight respectively. [a] $p < 0.05$, [b] $p < 0.025$, [c] $p < 0.005$ and [d] $p < 0.001$ with respect to control, analyzed by Student's t-test.

receptors such that an inhibitory influence on hypothalamic DA neurones is reduced and prolactin secretion inhibited. Our results are consistent with GABAergic modulation of dopaminergic transmission within the hypothalamus and with the observed changes in prolactin secretion, but they do not preclude effects of muscimol at GABAergic receptors which directly modulate prolactin secretion[20,21].

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Evidence against a role of $(Na^+ + K^+)$-ATPase in the alpha-adrenoceptor mediated positive inotropic effect of phenylephrine[1]

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Summary. Phenylephrine (0.1–100 μM) in the presence of 1 μM propranolol increased the force of contraction in electrically driven papillary muscles from cats. This presumably alpha-adrenoceptor mediated positive inotropic effect of phenylephrine occurred without any influence on $(Na^+ + K^+)$-ATPase activity.

It is generally accepted that the positive inotropic response to adrenergic agents in the heart is mediated predominantly by beta-adrenoceptors. However, evidence is increasing that alpha-adrenoceptors are also present in the myocardium and that positive inotropic effects may be produced by stimulation of these sites[3–5]. These 2 inotropic effects are qualitatively different from each other. The alpha-adrenergic positive inotropic response, for instance, appears to be independent of the cAMP-system[3–5]. In contrast, the increase in myocardial force of contraction due to alpha-adrenoceptor stimulation, but not that due to beta-adrenoceptor stimulation, is critically dependent on the frequency of stimulation[6–8]. Such a frequency-dependence has also been reported for cardiac glycosides[9] which, in turn, are widely believed to produce their positive inotropic effects via an inhibition of the $(Na^+ + K^+)$-ATPase activity[10]. With this in mind, the present experiments were designed to investigate whether an inhibition of $(Na^+ + K^+)$-ATPase activity might be involved in the alpha-adrenergic positive inotropic response. The effect of phenylephrine on myocardial force of contraction and on $(Na^+ + K^+)$-ATPase activity was studied under identical conditions. All experiments were performed in the presence of propranolol in order to minimize interference from beta-adrenoceptors.

Materials and methods. Cats (b.wt 0.8–2.0 kg) were anaesthetized with sodium pentobarbital (30 mg per kg i.p.) and papillary muscles (diameter 1 mm or less) were dissected from the right ventricles. The preparations were attached to a platinum stimulating electrode and mounted individually in glass tissue chambers for recording isometric contractions as described previously[11]. The bathing solution (50 ml) containing (mM) NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.5 was equilibrated with 95% O_2 + 5% CO_2 and maintained at 35 °C, pH 7.4. The preparations were driven electrically at a frequency of 0.2 Hz (duration 5 msec, intensity about 10% above threshold). Drugs used were (−)-phenylephrine HCl (Boehringer Ingelheim) and (±)-propranolol HCl (ICI). The compounds were freshly dissolved in bathing medium. Phenylephrine concentration-response curves were obtained cumulatively; the time of exposure to each concentration was 15 min. Propranolol (1 μM) was added 30 min before phenylephrine and was present during the entire experiment. We have shown previously[5] that beta-adrenoceptors are sufficiently blocked under these conditions.

After the papillary muscles had been dissected from the hearts, the remaining ventricular tissue was frozen and kept at −60 °C until used. After thawing, $(Na^+ + K^+)$-ATPase (EC 3.6.1.3) was prepared as described by Pitts and Schwartz[12] but without glycerol treatment. Enzyme activity was determined by the coupled optical assay in an ATP-regenerating system[13]. The incubation medium (buffered with $NaHCO_3$ to pH 7.4) was the same as that used for the