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DIFFERENTIAL SENSITIVITY OF HYPOTHALAMIC DOPAMINERGIC AND NORADRENERGIC NEURONES TO PHARMACOLOGICAL MANIPULATION

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The effects of apomorphine (Apo), haloperidol (Hal), reserpine, phenoxybenzamine, oxotremorine and scopolamine on hypothalamic catecholamines and metabolites were assessed. All these drugs, except Apo, significantly changed the hypothalamic concentration of 3-methoxy-4-hydroxyphenylglycol (MHPG), thus suggesting parallel changes in noradrenaline (NA) metabolism and turnover. Hal increased MHPG, an effect which was reversed by Apo pretreatment. Oxotremorine and scopolamine respectively increased and decreased MHPG, reserpine decreased NA and increased MHPG. Phenoxybenzamine increased MHPG without altering NA concentrations. Dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were not changed by Apo and Hal, but were influenced by the other drugs. These results indicate that NA in the hypothalamus is influenced by both cholinergic and dopaminergic events occurring in the brain and that dopaminergic neurones in this organ are different in their biochemical and pharmacological characteristics from neurones present in other central and peripheral systems.

Introduction Several anterior pituitary functions are believed to be tonically regulated by hypothalamic dopamine neurones, especially by those belonging to the tuberoinfundibular system (MacLeod, Fontham & Lehmeyer, 1970; Meites, 1977). Among the evidence sited in support of this, are the prominent effects of dopamine agonists and antagonists on the secretion of hormones such as prolactin (Meites, 1977). The median eminence is thought to be the area from which most hypothalamic releasing factors (both stimulatory and inhibitory) are secreted and transported (via a portal vessel system) to the pituitary. For example, the release of dopamine from the median eminence is believe to inhibit prolactin secretion (Meites, 1977). However, since the median eminence receives both dopaminergic and noradrenergic projections (Fuxe & Hökfelt, 1969), the role of noradrenaline (NA) in the secretion of pituitary hormones requires clarification. One approach to acquiring this information is to evaluate hypothalamic NA turnover and metabolism after treatments with drugs known to influence pituitary secretions via the release of median eminence dopamine.

In this communication we describe the effects of five drugs, apomorphine (Apo), haloperidol (Hal), phenoxybenzamine, reserpine, oxotremorine, and scopolamine on the concentration of catecholamines and their metabolites in the hypothalamus. Most of these drugs were reported to alter prolactin (Meites, 1977) secretion, therefore they are expected, at least theorotically to influence either directly or indirectly hypothalamic dopaminergic neurones.

Methods Male Sprague-Dawley rats weighing between 150 and 200 g were used. The dose and drug schedules followed are given in the footnotes to Table 1. Rats were killed by decapitation and the hypothalami removed and frozen on solid CO_2. These tissues were homogenized in 0.5 ml of 1 N HCl containing deuterated isomers of dopamine ([$^2H_5$]-DA), NA ([$^2H_3$]-NA), 3,4-dihydroxyphenylacetic acid (DOPAC) ([$^2H_5$]-DOPAC) and homovanillic acid (HVA) ([$^2H_2$]-HVA) at concentrations of 100 ng/ml. The homogenates were centrifuged and the clear supernatant transferred and stored at −30°C until analyzed. Total protein determination was carried out on the remaining pellet (Lowry, Rosebrough, Farr & Randall, 1951). Noradrenaline, dopamine and metabolites were analyzed by mass fragmentography as previously described (Karoum, Gillin, Wyatt & Costa, 1975; Karoum, Garrison, Neff & Wyatt, 1977a; Karoum, Noyer-Schwing, Potkin & Wyatt, 1977b) using a Finnigan model 4000 quadrupole gas chromatograph mass spectrometer. The amines and their metabolites were calculated by comparing their peak heights with those of the appropriate deuterated isomers.

All statistical comparisons were performed by two tailed *t* test. Controls were included in every batch of analysis in which one or more drugs were tested. The results obtained were then compared. The results of the controls shown in Table 1 were those representing the controls of rats used during treatments with Apo and Hal. These are typical of those obtained in other experiments.

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Results The changes observed in the hypothalamic concentration of catecholamines and their metabolites after various pharmacological manipulations are summarized in Table 1. The drugs employed were selected because of their relatively specific actions on catecholamines.

Haloperidol significantly elevated the concentration of MHPG but failed to alter the content of either dopamine or its metabolites. Apomorphine produced no change in any of the compounds assayed. However, pretreatment with it abolished the increase in MHPG induced by Hal.

Phenoxybenzamine increased MHPG and DOPAC, but not HVA. Reserpine (an inhibitor of catecholamine granular storage) depleted NA and DA, and increased MHPG concentration. Reserpine failed to change DOPAC, but significantly reduced HVA.

The cholinomimetic drugs, oxotremorine (muscarinic receptor agonist) and scopolamine (muscarinic receptor antagonist) respectively increased and decreased hypothalamic MHPG. Oxotremorine also increased dopamine and DOPAC concentrations while scopolamine decreased only dopamine. Therefore stimulation of brain muscarinic receptors appears to produce an increase in both dopamine and NA metabolism and possibly their turnover.

The mechanisms by which Hal and oxotremorine increased hypothalamic MHPG do not appear to be related. This is because pretreatment with Apo failed to antagonize the increase in MHPG induced by oxotremorine and combined scopolamine and Hal treatment produced an increase in MHPG similar to that caused by Hal alone. Apo did not antagonize the effect of oxotremorine on dopamine and DOPAC concentrations observed after oxotremorine alone (Table 1).

Discussion In spite of their extensive use in many investigations related to central and peripheral catecholamines, the effects of the above mentioned drugs (except phenoxybenzamine and reserpine) on hypothalamic NA turnover and metabolism have not been studied before. Furthermore, as far as we are aware, the concurrent effects of these drugs on hypothalamic dopamine and NA metabolism have not previously been evaluated. A concurrent evaluation can offer better insight into the role of catecholamines in hypothalamic neuroendocrine regulation of the pituitary than would be deduced from just one amine. For example, all the drugs we have studied excluding scopolamine, were reported to influence prolactin secretion (Meites, 1977) apparently through the release of hypothalamic dopamine (Perkins, Westfall, Paul, MacLeod &

Table 1 Concentration (mean ± s.e. mean) of hypothalamic catecholamines and metabolites (ng/mg protein) after drug treatments

Treatment (dose) (n)	*NA*	*MHPG*	*DA*	*DOPAC*	*HVA*
Saline control (8)†	44.9 ± 1.9	3.8 ± 0.25	9.4 ± 0.6	8.0 ± 0.7	6.5 ± 0.3
Oxotremorine (2.5 mg/kg) (5)	37.7 ± 1.5**	7.3 ± 0.3***	12.8 ± 1.6**	11.5 ± 2.2*	6.3 ± 0.6
Apomorphine (5 mg/kg) (9)	45.3 ± 5.7	4.5 ± 0.4	10.6 ± 0.8	10.2 ± 1.0	5.8 ± 0.5
Haloperidol (1 mg/kg) (8)	50.3 ± 5.8	6.7 ± 0.8***	9.1 ± 0.8	10.9 ± 0.0	6.4 ± 1.2
Phenoxybenzamine (20 mg/kg) (5)	36.8 ± 6.6	6.8 ± 0.9***	8.0 ± 0.7	12.6 ± 0.4***	5.3 ± 0.4
Reserpine (5 mg/kg) (8)	9.2 ± 1.0**	7.4 ± 2.4***	0.6 ± 0.1***	7.8 ± 1.3	3.3 ± 0.8***
Scopolamine (20 mg/kg) (4)	41.3 ± 2.0	2.8 ± 0.3**	5.5 ± 0.8***	8.2 ± 0.4	4.8 ± 0.7**
Combined apomorphine and haloperidol (5)	—	4.9 ± 0.8	—	—	—
Combined apomorphine and oxotremorine (5)	56.4 ± 4.2**	6.4 ± 0.3***	12.4 ± 0.8***	14.4 ± 2.6***	6.5 ± 1.1
Combined haloperidol and scopolamine (5)	—	8.7 ± 1.0***	—	—	—

For single dose treatments, the drugs were administered intraperitoneally for haloperidol, phenoxybenzamine, scopolamine, and subcutaneously for apomorphine, oxotremorine and reserpine. Rats were killed 15 min after oxotremorine, 45 min after apomorphine, 90 min after haloperidol and phenoxybenzamine and 2 h after reserpine. For the combined drug treatments; apomorphine (5 mg/kg) was administered at 0 and 45 min, haloperidol (1 mg/kg) at 15 min and the rats killed at 105 min; or haloperidol (1 mg/kg) at 0 min, scopolamine (20 mg/kg) at 30 and 60 min and the rats killed at 90 min; or apomorphine at 0 and oxotremorine at 30 min and rats killed at 90 min. All drugs in the combined treatments were administered by the same route as in the single dose treatments.

† These controls correspond to the apomorphine and haloperidol experiment. They are typical of the controls of other experiments.

*$P < 0.05$ compared with appropriate controls; **$P < 0.02$ compared with appropriate controls and ***$P < 0.005$ compared with appropriate controls by independent t test.

Rogol, 1979), yet as illustrated in Table 1, these drugs also affect hypothalamic NA. The possibility therefore may exist that pituitary secretions currently attributed to dopamine alone including prolactin are, at least partially, regulated by NA. Obviously the changes in NA metabolism observed here may be coincidental and as such, may have no relationship to any of these hormones. Furthermore, since in the present study we did not measure any pituitary hormone, the indirect influence of changes in these hormones release on NA turnover and metabolism should also be considered.

MHPG is the major NA metabolite in the rat brain (Schanberg, Schildkraut, Breese & Kopin, 1968, Karoum *et al.*, 1977b) and its concentration is directly related to NA turnover (Eccleston & Ritche, 1973; Stone, 1973). Similarly, DOPAC and HVA concentrations also reflect dopamine turnover within the brain (Karoum, Neff & Wyatt, 1977). The increase in MHPG concentration observed after Hal, phenoxybenzamine, reserpine and oxotremorine therefore may indicate increased NA turnover. Phenoxybenzamine has been found to increase NA turnover by others (Schanberg, *et al.*, 1968; Stone, 1976). This drug together with oxotremorine probably also increases dopamine turnover in the hypothalamus (DOPAC concentration was increased).

The results summarized in Table 1 illustrate several functional characteristics of noradrenergic nerve terminals in the hypothalamus. Interactions between brain cholinergic (both hypothalamic and extra-hypothalamic) and dopaminergic axons and hypothalamic NA nerve terminals are evident from the changes in MHPG induced by cholinomimetic and dopaminergic drugs (Table 1). Apparently, the increase in MHPG produced by blockade of dopamine receptors (Hal) and that by stimulation of muscarinic cholinoceptors (oxotremorine) are mediated by different mechanisms.

The changes in dopamine and its metabolites observed after reserpine appears characteristic of the hypothalamus. As shown here and previously found by others (Argiolas, Paghietti, Fadda, Pellegrini, Quarantotti & Gessa, 1978) reserpine reduced dopamine concentrations without stimulating its metabolism as seen in the caudate (unpublished) or in the sympathetic ganglion (Karoum, Speciale & Wyatt, 1979).

In conclusion, we have described the effects of several dopaminergic, adrenergic and cholinergic drugs on hypothalamic NA and dopamine metabolism and turnover. Hypothalamic NA metabolism was found to be sensitive to pharmacological manipulations by drugs which stimulate to block brain dopamine receptors or metabolism. From the results obtained, it was revealed that NA may play a role in the regulation of pituitary functions currently attributed to dopamine.

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