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Evidence for a prime role of newly synthesized dopamine in mesolimbic dopamine areas*

Enhancement of dopamine synthesis in the brain following administration of haloperidol, phenothiazines or other antipsychotic drugs has been demonstrated by several investigators (Andén, Roos & Werdinius, 1964; Laverty & Sharman, 1965; O'Keefe, Sharman & Vogt, 1970). While it has been shown by Besson, Cheramy & others (1973) that newly synthesized dopamine is released during impulse flow in the nigrostriatal axon, the relative functional importance of stored versus newly synthesized dopamine in brain function has been unclear.

Recently, a report from this laboratory presented evidence that in striatal function newly synthesized dopamine is utilized preferentially to stored dopamine (Shore & Dorris, 1975). In that study it was shown that a small dose of haloperidol, which alone caused only a slight degree of catalepsy, rapidly produced profound catalepsy when given to rats soon after administration of the tyrosine hydroxylase inhibitor, α-methyl tyrosine (α-MT). At the time of marked catalepsy potentiation about 80% of striatal dopamine was shown by analysis to be still present, suggesting that after haloperidol alone, the nigrostriatal system is functionally dependent largely on newly synthesized dopamine and that the main pool of the amine contributes little to the maintenance of striatal function. Analysis of striatal homovanillic acid (HVA) after the various treatments showed that the marked haloperidol-induced elevation of this extraneuronal dopamine metabolite was greatly inhibited by the presence of α-MT, a finding which is in accord with the concept that little of the main dopamine pool could have been released into the synaptic cleft. It was thus concluded that newly synthesized dopamine has a greater role in striatal function than does the large endogenous pool, at least under conditions of compensatory activation of the striatal dopamine neuron following dopamine receptor inhibition by a neuroleptic.

The present study examines another dopamine system, that of the mesolimbic system. The results suggest that a high degree of functional dependence on newly synthesized dopamine exists in the mesolimbic dopamine system as well as in the striatal system.

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Female Sprague-Dawley rats, 180–220 g were given α-methyltyrosine (50 mg kg^{-1}, i.p.). Some were also given haloperidol (1 mg kg^{-1}, s.c.) 30 min later. Other rats received haloperidol only. All rats were killed 90 min after haloperidol or 2 h after α-MT. Brains were removed rapidly, chilled, and the olfactory tubercle and nucleus accumbens were removed and combined for analysis as representative of the dopamine mesolimbic system. Tissues from six rats were pooled for each assay of either dopamine (determined by the method of Neff & Costa, 1966) or HVA (extracted as described by Murphy, Robinson & Sharman, 1969, and assayed by the method of Andén, Roos & Werdinius, 1963).

As reported elsewhere (Andén & Stock, 1973), haloperidol caused a marked rise in mesolimbic HVA. However, as with the striatal system, when rats were pretreated with α-MT, the HVA elevation was blocked (Table 1, I).

Table 1. *Effect of α-methyltyrosine: (I) on haloperidol-induced elevation of mesolimbic HVA concentration, (II) on mesolimbic dopamine concentration, (III) alone or replaced by haloperidol, or a combination of both, on mesolimbic dopamine concentration (n = no. of experiments).*

I			II			III		
Treatment		HVA ($\mu g\ g^{-1} \pm$ s.e.)	Time after α-MT		Dopamine ($\mu g\ g^{-1} \pm$ s.e.)	Treatment		Dopamine ($\mu g\ g^{-1} \pm$ s.e.)
Normal	(n=3)	0·14±0·06	0	(n=6)	6·49±0·36	Normal	(n=6)	6·49±0·36
Haloperidol	(n=4)	0·85±0·06*	30 min	(n=4)	5·11±0·19	Haloperidol	(n=3)	5·79±0·28
α-MT + haloperidol	(n=4)	0·07±0·07	60 min	(n=4)	4·67±0·33	α-MT	(n=3)	3·51±0·05†
			120 min	(n=3)	3·51±0·05	α-MT + haloperidol	(n=3)	2·86±0·20†

* $P < 0{\cdot}001$, † $< 0{\cdot}05$.

I. Haloperidol (1 mg kg^{-1}, s.c.) was given to rats, some of which had been treated with D,L-α-MT (50 mg kg^{-1}, i.p.) 30 min before. Animals were killed 90 min after haloperidol. Mesolimbic areas (olfactory tubercle and nucleus accumbens) from 6 rats were pooled for each experiment.

II. Rats were given D,L-α-MT (50 mg kg^{-1}, i.p.) and were killed at various times thereafter. Mesolimbic areas from 4 to 6 rats were pooled for each experiment.

III. Rats were given D,L-α-MT (50 mg kg^{-1}, i.p.). Some were also given haloperidol (1 mg kg^{-1}, s.c.) 30 min later. Other rats were given haloperidol alone. The rats were killed 90 min after haloperidol or 2 h after α-MT. Mesolimbic areas from 4 to 6 rats were pooled for each experiment.

Analysis of the mesolimbic areas showed a considerable normal dopamine content (about 6·5 $\mu g\ g^{-1}$). This concentration compares with about 12 $\mu g\ g^{-1}$ in the corpus striatum of rats of the same strain. Blockade of its synthesis caused a decline in its concentration approaching 50% over a 2 h period (Table 1, II). Haloperidol alone for 90 min had no significant effect on mesolimbic dopamine content (Table 1, III), but did further somewhat the decline caused by α-MT.

In the present study, no direct behavioural correlate could be studied in conjunction with neuroleptic effects on dopamine receptors in the mesolimbic areas in the manner that catalepsy was utilized as a behavioural correlate in the earlier study of striatal function. However, since HVA appears to be a measure of extraneuronal dopamine metabolism (Roffler-Tarlov, Sharman & Tegerdine, 1971) and is thus an index of the amine released into the synaptic cleft, the biochemical findings described in this study would seem to reflect adequately the degree of dopamine release from the nerve

terminal following dopamine receptor blockade by a neuroleptic. This is especially so since the current findings on dopamine mesolimbic areas parallel closely those reported earlier regarding the striatum.

In both dopamine systems the marked elevation of HVA caused by haloperidol was greatly inhibited by the simultaneous blockade of tyrosine hydroxylase, despite the continued presence of considerable quantities of dopamine remaining in the large endogenous pools. The evidence indicates that, in the mesolimbic centres, as well as in the striatum, by far the greater portion of dopamine released into the synaptic cleft after dopamine receptor blockade must arise from newly synthesized rather than from main store dopamine. In both systems, then, the newly synthesized amine appears to have the greater functional importance, at least under conditions of reflex activation of the dopamine receptor blockade.

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