The relative functional availability of brain noradrenaline and dopamine storage pools*

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A number of studies, carried out mainly by isotope release methods, have indicated that newly-synthesized brain noradrenaline and dopamine are preferentially released by nerve stimulation (Glowinski, 1970; Schildkraut, Draskoczy & Lo, 1971; Besson, Cheramy & others, 1973) and have a more rapid turnover than amines stored for a longer time (Thierry, Blanc & Glowinski, 1970; Javoy & Glowinski, 1971). Such evidence has led to the concept of small releasable pools along with larger storage pools of noradrenaline and dopamine.

In the dopaminergic system there is considerable direct evidence for a functional differentiation between two pools of dopamine, with the small releasable pool (representing mainly newly-synthesized amine) having a much greater significance than does the larger storage pool. For example, a behaviourally subeffective dose of haloperidol, if given after an inhibitor of tyrosine hydroxylase, exerts marked behavioural actions such as catalepsy and inhibition of operant behaviour (Ahlenius & Engel, 1971; Costall & Naylor, 1974; Shore & Dorris, 1975). Marked behavioural potentiation is seen even when the small dose of neuroleptic is given only a short time after synthesis blockade; for example, a profound potentiation of catalepsy is evident when most of the normal brain dopamine concentration (80%) remains present (Shore & Dorris, 1975). It would therefore appear that following a small dose of neuroleptic alone, sufficient dopamine can be released to overcome the actions of the neuroleptic, and that the origin of the mobilized dopamine must have been from the small pool of new dopamine, the large pool being relatively inert.

A similarly dramatic demonstration of the prime functional role of new dopamine can be seen in biochemical studies. The considerable rise in striatal or mesolimbic metabolites of dopamine normally appearing after treatment with haloperidol is not seen if tyrosine hydroxylase is inhibited even shortly before neuroleptic administration (Sears & Shore, 1975; Shore & Dorris, 1975), again despite the presence of large stores of dopamine. Thus it appears that stored dopamine can, at best, be transferred only slowly to the releasable pool.

We have now attempted to determine whether the central noradrenergic neuron operates by a similar mode. Biochemical studies analagous to those on the dopamine system were carried out. Specifically, the elevation of the rat brain noradrenaline metabolite, 3-methoxy-4-hydroxyphenylethylene glycol sulphate (MOPEG-SO_4) was measured following administration of centrally active α-adrenoceptor blocking drugs in the absence of, and following a short pretreatment with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine.

Female Sprague-Dawley rats, 200–240 g, were given (±)-α-methyl-*p*-tyrosine (α-MT) (Regis Chemicals) (100 mg kg^{-1}, i.p.). Some rats also were treated with either phenoxybenzamine (Smith, Kline & French) (40 mg kg^{-1}, i.p.) or clozapine (Sandoz) (12 mg kg^{-1}, i.p.) 30 min later. All rats were killed 60 min after the α-antagonist or 90 min after α-MT. Brains were rapidly removed, chilled in cold saline and kept frozen on dry ice until assayed the same day for MOPEG-SO_4 (Meek & Neff, 1972) or noradrenaline (Neff & Costa, 1966). Clozapine, either alone or in combination with α-MT, caused a 2° decrease in rectal temperature, an effect which did not occur with phenoxybenzamine.

As shown in Table 1, both phenoxybenzamine and clozapine caused a considerable rise in MOPEG-SO_4, an effect described by others (Keller, Bartholini & Pletscher, 1973; Braestrup & Nielsen, 1976). Treatment with α-MT caused only a slight, insignificant lowering of the metabolite. Administration of phenoxybenzamine or clozapine 30 min after α-MT effected

Table 1. *Effect of α-MT and α-adrenoceptor blockers and their combination on whole brain MOPEG-SO_4 and noradrenaline content.* Rats were injected with (±)-α-methyl-*p*-tyrosine (100 mg kg^{-1}, i.p.). Some rats were also given either phenoxybenzamine (40 mg kg^{-1}, i.p.) or clozapine (12 mg kg^{-1}, i.p.) 30 min later. Animals were killed 60 min after phenoxybenzamine or clozapine. Numbers in parentheses represent the number of animals in each group. The percent increase is of MOPEG-SO_4 concentrations with respect to the saline or α-MT controls.

	MOPEG-SO_4	% increase	Noradrenaline
	μg g^{-1} ± s.e.m.		μg g^{-1} ± s.e.m.
Saline	0·157 ± 0·004 (20)		0·490 ± 0·014 (8)
Phenoxybenzamine	0·231 ± 0·006* (6)	47	
Clozapine	0·258 ± 0·017* (6)	64	
α-MT	0·143 ± 0·008 (6)		0·380 ± 0·010 (8)
α-MT + phenoxybenzamine	0·240 ± 0·016* (6)	68	0·308 ± 0·008* (8)
α-MT + clozapine	0·224 ± 0·006* (6)	57	0·323 ± 0·014* (8)

* Differs from saline or α-MT control, $P < 0·01$ (Dunnett's *t*-test).

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increases in MOPEG-SO_4 to about the same extent as in the rats without α-MT. A decrease in noradrenaline concentration was seen after α-MT. The addition of either drug caused a further lowering.

These results contrast strikingly with those seen on the dopamine system as described above. It would appear that, unlike the dopamine system, both newly-synthesized and stored noradrenaline are readily available for neurogenic release at least under conditions of heavy demand such as after noradrenaline receptor blockade. Stone (1976) has reported that α-MT pretreatment did not affect the appearance of metabolites of noradrenaline in the hypothalamus of the rat until noradrenaline concentrations were depleted by 40%. In our short-term experiments, α-MT lowered brain noradrenaline only 22% while even with the additional administration of phenoxybenzamine or clozapine there resulted a total depletion of less than 40%. Our results, and those of Stone (1976), suggest that with the noradrenaline system, exchange between storage and functional sites readily occurs or, alternatively, the functional pool represents a very large proportion of the total noradrenaline content. However, it seems unlikely that the newly-synthesized pool represents more than 20% of the total noradrenaline concentration (Sedvall, Weise & Kopin, 1968; Thierry, Blanc & Glowinski, 1970). Another possibility is that where isotopic studies have indicated preferential release of new noradrenaline this results from releasable amine being synthesized or taken up primarily by vesicles in close proximity to the synaptic cleft, a geometric aspect previously considered (see discussion, Glowinski, 1970). If this is so, then truly separate pools of endogenous noradrenaline may not exist in central noradrenergic neurons.

Franklin & Herberg (1975), have reported findings using a rat behavioural model that provides an interpretation of the central noradrenaline system consistent with the above biochemical findings. They reported that inhibition of dopamine-β-hydroxylase with FLA-63 [bis(4-methyl-1-homopiperazinylthiocarbonyl)disulphide] did not inhibit self-stimulation of brain noradrenergic areas, but that pretreatment with reserpine three to five days before FLA-63 severely depressed self-stimulation rates. This evidence also indicates the important functional role of stored noradrenaline in the central noradrenaline system.

This interpretation assumes that phenoxybenzamine releases noradrenaline by blockade of the adrenoceptors to remove the normal autoinhibition mechanisms that control release. It also assumes that the proportion of the released noradrenaline that ends up as MOPEG-SO_4 remains unaltered under the different experimental conditions. It seems unlikely that the phenoxybenzamine in these experiments acts at any other site than the adrenoceptors (although it has been shown to affect other receptors in concentrations higher than those needed for block of adrenoceptors in *in vitro* preparations for example). Moreover, each blocking drug has been used as its own control, and both had the same effect on MOPEG-SO_4 whether or not noradrenaline synthesis had been inhibited. Regardless of the exact site where either phenoxybenzamine or clozapine exerted their effects, there is little reason to suppose that it would be different after the inhibition of noradrenaline synthesis.

In conclusion, the accumulated evidence argues that in the dopamine neuron there exists a marked functional difference between two demonstrable dopamine pools while in the noradrenaline neuron there is little or no functional difference if, in fact, truly separate pools exist.

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