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Effect of adrenergic neuron blocking agents and biguanides on the efflux of extragranular noradrenaline from adrenergic nerves in rabbit atria

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The efflux of (—)-[^{3}H]noradrenaline from reserpine- and pargyline-pretreated rabbit atria was accelerated by phenethylamine and tryptamine derivatives, possibly as a result of accelerative exchange diffusion (Paton, 1973a, 1975; Paton & Pasternak, 1974). The most potent phenethylamines studied were β-phenethylamine, (+)- and (—)-amphetamine and phentermine (Paton, 1975). All the tryptamine derivatives studied were much less potent (Paton, 1973a). The potency of phenethylamines and tryptamines was reduced by hydroxylation or *o*-methylation on the ring, by β-hydroxylation and by *N*-substitution. In the present study, we have examined the effects of adrenergic neuron blocking agents and related biguanides on the efflux of extragranular (—)-[^{3}H]noradrenaline in order to further elucidate the structural requirements for acceleration of efflux.

As described previously (Paton, 1973b), atria, from reserpine pretreated rabbits, were exposed to pargyline (5×10^{-4}M for 30 min) and tropolone (10^{-4}M throughout), and thereafter to 10^{-7}M or 5×10^{-7}M (—)-[^{3}H] noradrenaline for 60 min (in the continued presence of tropolone). Tissues were then blotted and transferred every 5 min to fresh media at 37°. Drugs were added between 60–90 min of efflux because, during this period, efflux occurs predominantly from adrenergic nerves (Paton, 1973b). Tropolone was present throughout the efflux. At 90 min tissues were removed, blotted and the [^{3}H]noradrenaline remaining in the tissues (in pmol g^{-1} wet weight) was then determined.

It can be seen (Table 1) that, at 10^{-4}M, all the adrenergic neuron blocking agents studied increased the efflux of (—)-[^{3}H]noradrenaline significantly, the most potent agents being bretylium and guanethidine. However, all were less potent than *p*-tyramine. The biguanidines, metformin (*NN*-dimethyldiguanide) and phenformin (phenethyldiguanidine), were inactive at 10^{-4}M. The adrenergic neuron blocking agents had significantly less effect on efflux at less than 10^{-4}M.

The effects on efflux of β-phenethylamine, (±)-phenylethanolamine and (±)-β-hydroxyphenethylguanidine were also examined (Table 2). This study illustrated the tendency for potency to be reduced by β-hydroxylation as noted previously (Paton, 1975), and the marked additional reduction in potency resulting from the guanidine substitution on the terminal nitro-

Table 1. *Effect of adrenergic neuron blocking agents and biguanides on the efflux of* (—)-[^{3}*H*]*noradrenaline.* Tissues were exposed to 10^{-7}M (—)-[^{3}H]noradrenaline before efflux. All drugs were present from 60–90 min of efflux at 10^{-4}M. Mean ± s.e. of 5–14 observations. *$P<0.05$ (compared to control value).

Compound	Residual (—)-[^{3}H]noradrenaline content (pmol g^{-1})
—	257 ± 24
Metformin	268 ± 39
Phenformin	222 ± 33
Debrisoquin	177 ± 25*
β-Hydroxyphenyl-ethylguanidine	175 ± 18*
Guanochlor	166 ± 17*
Guanoxon	151 ± 20*
Guanethidine	137 ± 23*
Bretylium	126 ± 28*
p-Tyramine	106 ± 15*

Table 2. *Effect of phenethylamine derivatives on the efflux of* (—)-[^{3}*H*]*noradrenaline.* Tissues were exposed to 5×10^{-7}M (—)-[^{3}H]noradrenaline before efflux. All drugs were present from 60–90 min of efflux at the concentrations indicated. Mean ± s.e. of 6 observations. *$P<0.05$ (compared to control values).

Compound	Concentration (M)	Residual (—)-[^{3}H] noradrenaline content (pmol g^{-1})
—	—	2617 ± 444
β-Phenethylamine	5×10^{-6}	1069 ± 181*
	5×10^{-5}	1017 ± 179*
(±)-Phenylethanolamine	5×10^{-6}	1552 ± 288*
	5×10^{-5}	1003 ± 173*
(±)-β-Hydroxyphenylethyl-guanidine	5×10^{-6}	2332 ± 290
	5×10^{-5}	2533 ± 507

gen. The effect of β-phenethylamine on efflux was near-maximal at 5×10^{-6}M confirming earlier results (Paton, 1975).

These findings have demonstrated that adrenergic neuron blocking agents accelerate the efflux of (—)-[^{3}H]noradrenaline located in the cytoplasm of adrenergic neurons. This effect cannot result from inhibition of re-uptake of amine (i.e., by competitive exchange diffusion) since cocaine and desipramine did not alter efflux (Paton, 1973b). Rather these agents may act by displacing noradrenaline from postulated cytoplasmic binding sites for noradrenaline or by accelerative exchange diffusion (Paton, 1975).

These findings have also shown that neither a phenyl ring nor a terminal amino group separated from the ring by two carbon atoms, are necessary for activity since a variety of structures were capable of accelerating the efflux of (—)-[^{3}H]noradrenaline. However, optimal activity requires this structure (Paton, 1973a; 1975) as is also the case for uptake in adrenergic neurons (Ross, 1976). A number of adrenergic neuron blocking agents (e.g., guanethidine, bretylium, bethanidine) appear to be transported into adrenergic nerves by the same carrier utilized by noradrenaline (Ross, 1976).

It is interesting that the biguanides, metformin and phenformin, had no effect on efflux. These compounds inhibited sugar and amino acid transport in intestine (Caspary & Creutzfeldt, 1973) and renal cortical slices (Robinson & Luisier, 1973) possibly as a result of inhibiting energy metabolism and/or causing Na^+ enrichment. It seems unlikely that the diguanides produced these effects in the present study since metabolic inhibition and ouabain have been shown to markedly accelerate the efflux of (—)-[^{3}H]noradrenaline (Paton, 1973b).

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