Inhibition of noradrenaline uptake by drugs

SIR,—The *in vivo* studies of Axelrod, Whitby & Hertting (1961), Hertting, Axelrod & Whitby (1961), Hertting, Axelrod & Patrick (1962) and Axelrod, Hertting & Potter (1962) and the *in vitro* studies of Dengler, Spiegel & Titus (1961) showed that the uptake of noradrenaline into sympathetically innervated tissues can be inhibited by a wide variety of drugs. Among the compounds reported to act as inhibitors of noradrenaline uptake were cocaine, reserpine, guanethidine, imipramine, chlorpromazine and the adrenergic blocking agents dichloroisoprenaline (DCI), ergotamine and phenoxybenzamine. The present study was undertaken as a quantitative investigation of the potencies of these and certain other drugs as inhibitors of noradrenaline uptake.

In previous reports from this laboratory the kinetics of noradrenaline uptake in the isolated perfused rat heart and the inhibition of this process by a group of sympathomimetic amines have been described (Iversen, 1963; 1964). The methods used in the present experiments are described in these papers. Drugs were added to the perfusing medium, which also contained ^{14}C-noradrenaline. The uptake of noradrenaline was measured by analysing the ^{14}C-noradrenaline content of the heart at the end of a 10 min. perfusion. Each drug concentration was tested on a group of four hearts and the results were expressed as the mean percentage inhibition of noradrenaline uptake in the drug-treated group when compared with the uptake in drug-free controls. When sufficient data were available the drug concentration required to produce a 50% inhibition of noradrenaline uptake (ID50) was calculated.

TABLE I. THE INHIBITION OF NORADRENALINE UPTAKE BY DRUGS IN THE ISOLATED RAT HEART

Drug	Drug concentration (M)	% inhibition of noradrenaline uptake	Drug ID50 (M)
Desipramine	1×10^{-8}	43·5	
Desipramine	1×10^{-7}	81·0	$1{\cdot}3 \times 10^{-8}$
Desipramine	1×10^{-6}	92·5	
Imipramine	1×10^{-7}	45·5	
Imipramine	1×10^{-6}	75·0	$9{\cdot}0 \times 10^{-8}$
Bisdesipramine	1×10^{-7}	37·0	—
Reserpine	1×10^{-6}	73·0	—
Chlorpromazine	1×10^{-5}	88·5	—
Cocaine	1×10^{-7}	25·0	
Cocaine	1×10^{-6}	68·0	
Cocaine	1×10^{-5}	95·0	$3{\cdot}8 \times 10^{-7}$
Guanethidine	2×10^{-6}	41·0	
Guanethidine	2×10^{-5}	78·5	$3{\cdot}3 \times 10^{-6}$
Bretylium	2×10^{-6}	10·0	
Bretylium	2×10^{-5}	60·0	$1{\cdot}4 \times 10^{-5}$
Adrenergic blocking agents			
Phenoxybenzamine	1×10^{-5}	91·5	
DCI	2×10^{-6}	50·5	
Pronethalol	5×10^{-6}	36·0	
Phentolamine	1×10^{-5}	66·0	
Dibenamine	1×10^{-5}	34·0	
Monoamine oxidase inhibitors			
Tranylcypromine	1×10^{-5}	88·0	
Harmine	1×10^{-5}	79·5	
Phenelzine	1×10^{-5}	69·0	
Isocarboxazide	1×10^{-5}	14·5	
Nialamide	1×10^{-5}	3·5	
Pargyline	1×10^{-5}	nil	
Iproniazid	1×10^{-5}	nil	

The results obtained with 21 drugs are presented in Table 1. Cocaine and imipramine were very potent inhibitors of noradrenaline uptake. As reported by Titus & Spiegel (1962) the mono-*N*-methyl derivative of imipramine (desipramine) is an even more potent inhibitor of noradrenaline uptake than

imipramine. Desipramine is the most effective inhibitor of noradrenaline uptake so far described, it is approximately 30 times more potent than cocaine, 7 times more than imipramine and 6 times more than (–)-metaraminol (Iversen, 1964). The primary amine derivative of imipramine (bisdesipramine) was also tested but this compound proved to be less effective than desipramine.

The uptake of noradrenaline into tissues is thought to represent a major mechanism in the inactivation of noradrenaline. Drugs which act as inhibitors of this inactivation process should therefore have the property of potentiating the actions of noradrenaline on the smooth muscle receptor sites in sympathetically innervated tissues, since in the absence of tissue uptake more noradrenaline will be made available to interact with such receptor sites. This potentiation will include the effects of noradrenaline released from endogenous stores within the tissues by nerve impulses and the effects of noradrenaline introduced exogenously to the tissue. These potentiating effects are well known in cocaine and have also been reported in other inhibitors of uptake such as guanethidine and phenoxybenzamine (Stafford, 1963). The potent inhibitors of uptake, imipramine and desipramine are also particularly effective in potentiating the actions of noradrenaline (Sigg, Soffer & Gyermek, 1963). In agreement with this hypothesis, the present findings that desipramine is more potent than imipramine as an inhibitor of noradrenaline uptake agree with the pharmacological findings that desipramine is more effective than imipramine in potentiating the actions of noradrenaline.

With imipramine and desipramine it is possible that many of their pharmacological properties can be explained by their ability to prevent the inactivation of noradrenaline by tissue uptake. However, this is not so with most of the other compounds listed in Table 1. For example drugs whose major action is thought to be a blockade of either α- or β-adrenergic receptor sites were also found to act as inhibitors of noradrenaline uptake. Of the five adrenergic blocking agents tested all had some activity as inhibitors of uptake. In contrast to previous reports (Hertting & others, 1961; Lindmar & Muscholl, 1964) phentolamine and dibenamine were found to act as inhibitors of uptake, though these compounds were only weakly active. In the present experiments no correlation was apparent between the ability to block either α- or β-receptors and the ability to inhibit noradrenaline uptake, since both α- and β-blocking drugs were active as inhibitors of uptake. These results thus do not support the view that the inactivation of noradrenaline by tissue uptake involves an uptake or binding at either α- or β-adrenergic receptors (Kirpekar & Cervoni, 1963).

The present findings emphasise the importance of considering the possibility that a drug which affects adrenergic mechanisms may well have multiple sites of action. This point is also well illustrated by the results obtained when a group of monoamine oxidase inhibitors was tested. In addition to the ability to inhibit the enzyme monoamine oxidase, three of the seven drugs tested possessed the ability to inhibit noradrenaline uptake. Similarly guanethidine and bretylium were active as inhibitors of noradrenaline uptake, though it seems unlikely that this property can account for the adrenergic blockade produced by these compounds. In confirmation of the findings of Hertting, Axelrod & Patrick (1962) *in vivo*, bretylium was found to be approximately ten times less potent than guanethidine in inhibiting the uptake of noradrenaline.

Brodie & Beaven (1963) have proposed that the depleting action of reserpine on the tissue stores of noradrenaline can be explained by the inhibition of noradrenaline uptake produced by this drug. They suggested that reserpine inhibits an active accumulation of noradrenaline into adrenergic nerves, and

that in the absence of this uptake process the intracellular noradrenaline leaks out of the tissue by passive diffusional processes. The present results do not support this hypothesis, since there was no correlation between the ability to inhibit noradrenaline uptake and the ability to deplete the endogenous noradrenaline content of tissues among the compounds tested. Although reserpine and guanethidine are active as noradrenaline depleting agents and are also effective as inhibitors of uptake, there were several other equally potent inhibitors of noradrenaline uptake such as cocaine, imipramine and chlorpromazine, which are without effects on the levels of endogenous noradrenaline in tissues (Muscholl 1961; Gey & Pletscher, 1961).

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