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Inhibition of catecholamine uptake in the isolated rat heart by haloalkylamines related to phenoxybenzamine

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Summary

1. Twenty-one haloalkylamine derivatives were tested as inhibitors of both the neuronal uptake of ^{3}H-noradrenaline (NA) by the Uptake$_1$ mechanism and the extraneuronal uptake of ^{3}H-NA by the Uptake$_2$ mechanism in the isolated rat heart.

2. At a concentration of 50 μM most of the compounds tested caused a significant inhibition of both uptake processes, although there were wide differences in the relative effects on Uptake$_1$ and Uptake$_2$. Some tentative structure activity relationships for uptake inhibition were formulated from these results.

3. Phenoxybenzamine was confirmed to be a potent inhibitor of both the Uptake$_2$ and Uptake$_1$ mechanisms, with IC50 values for these two systems of 2·8 μM and 0·9 μM respectively.

4. The substances *N*-(9-fluorenyl)-*N*-methyl-β-chloroethylamine (SKF 550), *N*-(3,4-dimethoxyphenylisopropyl)-*N*-benzyl-β-chloroethylamine (SKF 625A) and *N*-(4-methoxyphenoxyisopropyl)-*N*-benzyl-β-chloroethylamine (SKF 784A) were significantly more potent than phenoxybenzamine as Uptake$_2$ inhibitors, and were all less potent than phenoxybenzamine as Uptake$_1$ inhibitors. The compound SKF 550 is the most potent and selective inhibitor of Uptake$_2$ so far described. It has an IC50 for Uptake$_2$ of 0·08 μM, and an IC50 for Uptake$_1$ of approximately 40·0 μM.

5. Comparison of the present results with the known activities of these blocking agents suggests that no correlation exists between adrenoceptor blocking activity and ability of the substances to act as inhibitors of Uptake$_2$ or Uptake$_1$.

Introduction

Catecholamines are accumulated by two different uptake mechanisms in the rat heart. There is an uptake of catecholamine into adrenergic nerve terminals (Uptake$_1$) and also an uptake of catecholamine into extraneuronal sites mediated by a second uptake mechanism, termed 'Uptake$_2$' (Iversen, 1971). Although a large number of potent inhibitors of the neuronal uptake mechanism are available, relatively few inhibitors of the Uptake$_2$ mechanism are known. The most potent inhibitors so far described for this mechanism are metanephrine (Burgen & Iversen, 1965), β-oestradiol (Iversen & Salt, 1970; Salt, 1972), and the haloalkylamine phenoxybenzamine (Lightman & Iversen, 1969). The latter compound is a potent inhibitor of Uptake$_2$ (IC50=approx. 2·5 μM; IC50=drug concentration required to produce 50% inhibition of catecholamine uptake) but has several

other actions. It is also a powerful inhibitor of the $Uptake_1$ mechanism in the rat heart (Iversen & Langer, 1969) with an IC50 for this effect of approximately 0·9 μM. In addition, phenoxybenzamine is a well-known α-adrenoceptor antagonist (Nickerson & Nomaguchi, 1951 ; Graham, 1962). In a search for more selective inhibitors of $Uptake_2$, a series of substances related to phenoxybenzamine were examined. In selecting these compounds, particular attention was paid to substances with structures similar to that of phenoxybenzamine, but with the addition of methoxy groups to various portions of the aromatic substituents, in the hope that such compounds might show increased activity as $Uptake_2$ inhibitors, as found in a series of phenylethylamine derivatives by Burgen & Iversen (1965). This prediction proved to be correct, and several substances are described which are considerably more potent and more selective than phenoxybenzamine as $Uptake_2$ inhibitors.

Methods

Perfusion technique

Male albino Wistar rats weighing 150–200 g were injected intraperitoneally with sodium pentobarbitone, 60 mg/kg, and heparin 1,000 units. Five minutes later the hearts were removed and perfused by the Langendorff technique as previously described (Iversen, 1963). The perfusion medium was a Krebs–Henseleit solution of the following composition, in g/l.: NaCl, 6·90 ; KCl, 0·35 ; $CaCl_2.2H_2O$, 0·37 ; KH_2PO_4, 0·15 ; $MgSO_4.7H_2O$, 0·29 ; $NaHCO_3$, 2·10 ; glucose, 2·0. To this was added EDTA disodium salt at a concentration of 10 mg/l., and ascorbic acid 20 mg/l. to reduce auto-oxidation of noradrenaline (NA) (Iversen, 1963). The Krebs solution was gassed with a mixture of 5% carbon dioxide in oxygen, and maintained at 37° C.

Effects of drugs on $Uptake_2$

After perfusion for 5 min with amine-free solution the hearts were perfused for a further 4 min with Krebs solution containing (±)-^{3}H-noradrenaline (Radiochemical Centre, Amersham, 7·0 Ci/mmol) diluted with non-radioactive (±)-NA to a final concentration of 5 μg/ml and 125 nCi/ml. Stock solutions of the phenoxybenzamine analogues were prepared immediately before use by dissolving the compounds in approximately 0·1 ml of ethanol containing a minimum amount of hydrochloric acid for solution. The stock solution was then diluted in a large volume (minimum 1,000 volumes) of Krebs solution containing ^{3}H-NA. After perfusion the hearts were removed, blotted free of perfusion medium and homogenized in 2 ml 1% EDTA ; 10 ml of acid ethanol was then added and the samples centrifuged. The radioactivity in 1 ml aliquots of the supernatant was measured in a Packard Tri-Carb liquid scintillation spectrometer after adding 4 ml ethoxyethanol and using 10 ml of 0·4% butyl PBD (CIBA) in toluene as the scintillator. Efficiency of counting was determined by the use of external standards. The tissue content of the labelled NA plus metabolites was calculated, and the results were corrected for the presence of ^{3}H-NA in the extracellular space of the tissue, assuming this to be 33% of the wet weight (Iversen, 1963). The concentration of NA in the extracellular fluid was considered equal to that in the perfusion medium, as the extracellular space equilibrates rapidly with the perfusate (Morgan, Henderson, Regen & Park, 1961). The extraneuronal uptake of NA ($Uptake_2$) was calculated after further correction for a predicted intraneuronal uptake ($Uptake_1$) of

^{3}H-NA of 0·8 μg/g per 4 min (Lightman & Iversen, 1969). Those drugs which in preliminary screening experiments produced a substantial inhibition of $Uptake_2$ were examined in more detail, and the percentage inhibition produced by at least four different concentrations of these drugs was determined. At each concentration groups of six hearts were perfused. A log-probit plot of the results for each drug was made, and the line of best fit obtained by the method of least squares. From this plot the concentration of drug producing 50% inhibition of $Uptake_2$ (IC50) was calculated and the standard error was determined using the graphical method of Miller & Tainter (1944).

Effect of drugs on $Uptake_1$

To test the specificity of action of phenoxybenzamine analogues, their ability to antagonize the uptake of NA by $Uptake_1$ into sympathetic nerve terminals was also checked. In these experiments, design was essentially the same as for the $Uptake_2$ experiments except that the hearts were perfused for 10 min with (±)-^{3}H-NA (Radiochemical Centre, Amersham, 7·0 Ci/mmol) diluted with non-radioactive (±)-NA to a final concentration of 10 ng/ml and 50 nCi/ml. There was no washout period at the end of the perfusion with NA, and a correction was made to the results to eliminate the contribution made by the presence of radioactivity in the extracellular space, as before.

Effects of drugs on the metabolism of ^{3}H-noradrenaline

In both series of $Uptake_1$ and $Uptake_2$ experiments, the extraction of ^{3}H-NA and its metabolites was sometimes modified in order to measure the accumulation of unchanged catecholamine into neuronal and extraneuronal sites respectively. ^{3}H-NA was separated from normetanephrine and from its deaminated metabolites by ion exchange chromatography as described by Iversen (1963). In these experiments the perfusion schedule was identical to that given above. The hearts were homogenized in 2 ml 1% EDTA and 10 ml 0·4 N perchloric acid and 10 mg ascorbic acid were added. The homogenate was centrifuged at 2,000 *g* in a bench centrifuge for 10 min and a 1·5 ml aliquot of the supernatant was counted to determine total radioactivity. The supernatant was then neutralized and chromatographed on a 5 cm column of Amberlite CG 120 (Na^+ form) resin as described by Iversen (1963). Samples of the eluates containing ^{3}H-NA and ^{3}H-normetanephrine (NMN) were counted using 10 ml of a 2:1 (v/v) solution of toluene:Triton-X-100 containing 0·4% butyl PBD as scintillant. Column recoveries for NA and NMN were determined and averaged 75% for each compound. A small amount of NA (less than 5%) was found to contaminate the NMN fraction but this was not corrected for in the calculations. By measuring total radioactivity, labelled NA and normetanephrine, it was possible to calculate the percentage of deaminated metabolites by differences.

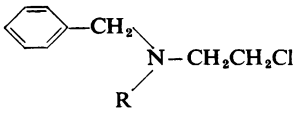
Results

Screening of phenoxybenzamine analogues as $Uptake_1$ and $Uptake_2$ inhibitors

Twenty-one haloalkylamines were tested for their ability to inhibit the uptake of ^{3}H-NA from low ($Uptake_1$) or high ($Uptake_2$) perfusion concentrations of NA in the isolated rat heart. In these experiments the hearts were exposed to ^{3}H-NA

and to the haloalkylamine simultaneously, and all drugs were tested initially at a concentration of 50 μM. The results (Tables 1–3) indicated that eighteen compounds produced a significant inhibition of $Uptake_2$ and twenty compounds inhibited $Uptake_1$. There were, however, considerable differences in the relative inhibitions of $Uptake_1$ and $Uptake_2$ by the various haloalkylamines. It was not possible to formulate precise structure activity relationships for $Uptake_1$ and $Uptake_2$ inhibition from these results, but certain features seem worthy of comment. For inhibition of $Uptake_1$, compounds may conveniently be compared with phenoxybenzamine (Table 1). The present results confirm previous findings that phenoxybenzamine is a potent inhibitor of $Uptake_1$ (Iversen & Langer, 1969). Other compounds with an *N*-phenoxyethyl substituent, such as SKF 519 and 513 produced an inhibition of $Uptake_1$ similar to that caused by phenoxybenzamine; the absence of a second aromatic substituent (e.g. SKF 519) had no effect on $Uptake_1$ inhibition. The compound SKF 195A, with an *N*-phenylethyl substituent was also particularly effective as an $Uptake_1$ inhibitor. On the other hand, dibenamine

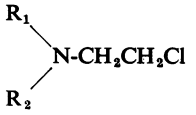
TABLE 1. *Inhibition of $Uptake_1$ and $Uptake_2$ in isolated rat heart by* N*-benzyl haloalkylamines*

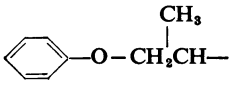
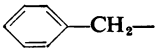
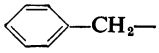
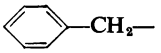
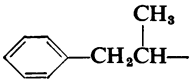
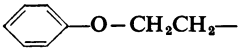
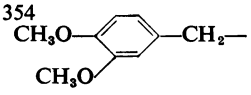
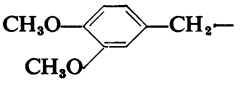
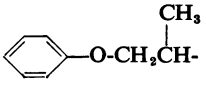
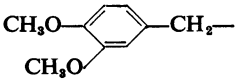
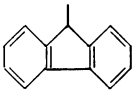
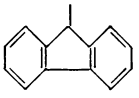


Compound	% Inhibition of ^{3}H-NA uptake: $Uptake_2$	$Uptake_1$
SKF 688A Phenoxybenzamine	88·0± 5·5	88·1± 2·2
SKF 199 Dibenamine	84·6± 6·6	37·6± 4·2
SKF 521	81·1±23·2	92·9± 1·3
SKF 353	56·8± 6·6	69·7± 2·8
SKF 195A	80·4± 1·2	98·1± 0·39
SKF 194A	79·1±11·6	45·8± 4·8
SKF 513	87·0± 1·9	98·1± 0·08
SKF 883A	84·1± 8·9	71·1± 3·4
SKF 784A	99·9± 1·3	50·9±14·4
SKF 625A	91·7± 3·3	29·0± 1·8

Drugs were tested at a concentration of 50 μM, and the percentage inhibition of $Uptake_1$ and $Uptake_2$ was determined in separate experiments, as described in **Methods**. The overall mean uptake of ^{3}H-NA in control perfusions was 0·21±0·01 μg/g per 10 min for $Uptake_1$ and 3·77±0·21 μg/g per 4 min for $Uptake_2$ (means±S.E.M. for 42 and 78 hearts respectively). Each value is the mean±S.E.M. for 4–12 hearts. These details apply also to the results in Tables 2 and 3.

TABLE 2. *Inhibition of Uptake$_1$ and Uptake$_2$ in isolated rat heart by haloalkylamines of formula:*



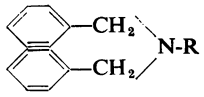
Compound	R_1	R_2	% Inhibition of ^{3}H-NA uptake: Uptake$_2$	Uptake$_1$
SKF 688A Phenoxybenzamine			88·0± 3·5	88·1±2·2
SKF 199 Dibenamine			84·6± 6·6	37·6±4·2
SKF 502		CH_3CH_2—	96·9±10·3	57·2±4·0
SKF 519		CH_3CH_2—	71·6± 5·3	90·7±1·1
SKF 354			38·3±18·4	89·4±1·8
SKF 880A			70·3± 3·1	80·7±1·3
SKF 550		CH_3—	79·5±10·9	36·0±3·9
SKF 560		$CH_3CH_2CH_2CH_2$—	32·2± 7·2	31·6±2·9

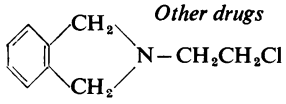
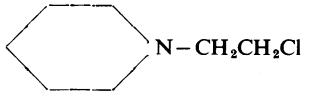
which has two *N*-benzyl substituents was much less active than SKF 195A. Methoxy substituents on the aromatic groups tended to reduce Uptake$_1$ inhibition, particularly if the methoxylation was of an *N*-phenylethyl or phenoxyethyl group (c.f. 625A with 194A ; 784A with phenoxybenzamine). Compounds 318 and 198 with substituents on the chloroethylamine chain which may hinder or prevent the formation of the ethyleneiminium ion retained Uptake$_1$ inhibitory activity. For Uptake$_2$ inhibition, methoxylation of the *N*-phenoxyethyl or phenylethyl substituents enhanced inhibitory activity (c.f. 625A with 194A ; 784A with phenoxybenzamine). Compounds 318 and 198 had reduced activity compared with dibenamine, suggesting a possible involvement of the ethyleneiminium ion in Uptake$_2$ inhibition.

IC50 values for Uptake$_2$ inhibition

Six compounds were selected for further study, and IC50 values were determined for these substances (Figs. 1, 2) (Table 4). The IC50 value for phenoxybenzamine of 2·82 μM is in good agreement with our previous findings (Lightman & Iversen, 1969). Dibenamine was approximately five times less potent than phenoxy-

TABLE 3. *Inhibition of Uptake$_1$ and Uptake$_2$ in isolated rat heart by haloalkylamines of formula:*



Compound	R =	% Inhibition of ^{3}H-NA uptake: Uptake$_2$	Uptake$_1$
SKF 198	$-CH_2CH_2Cl$ C_2H_5	6·0±20·3	93·0± 1·5
SKF 318	CH_2Cl $-C-CH_3$ CH_2Cl	36·0±12·5	83·7± 2·1
SKF 1037C	$-CH_2CH_2Br$	68·8± 1·0	24·8± 7·1
Other drugs			
SKF 317		21·2± 3·3	27·8±15·0
SKF 191		19·8± 9·8	38·4± 3·2

benzamine, and the compound SKF 880A, with methoxylation of the *N*-benzyl substituent, had approximately the same potency as phenoxybenzamine. The compounds SKF 784A and 625A, however, with methoxylation of *N*-phenoxyethyl or phenylethyl groups, were significantly more potent than phenoxybenzamine, and the substance SKF 550, with an *N*-fluorenyl substituent, was approximately 35 times more potent than phenoxybenzamine, and is the most potent inhibitor of Uptake$_2$ so far described. The slopes of the log-probit plots for Uptake$_2$ inhibition by these substances were similar (Table 4), with the exception of dibenamine and SKF 550 which gave plots of somewhat greater slope than the other compounds. This may possibly reflect a different mode of action of these drugs in inhibiting Uptake$_2$.

In some experiments, the more potent Uptake$_2$ inhibitors (SKF 625A and 550) were tested to determine whether they influenced the metabolism of ^{3}H-NA. In these studies hearts were perfused with various concentrations of the inhibitor, as before, and the tissue extracts were then subjected to ion exchange chromatography

TABLE 4. *IC50 values for Uptake$_2$ inhibition by haloalkylamines*

Drug	IC50 value (μM)	Slope of IC50 plot±S.D. (probit/log concentration)
SKF 550	0·08±0·02	2·89±0·14
SKF 625A	0·25±0·09	1·98±0·22
SKF 784A	1·07±0·37	2·07±0·13
Phenoxybenzamine	2·82±1·47	1·60±0·23
SKF 880A	2·65±1·62	1·47±0·10
Dibenamine	14·90±2·85	3·47±0·23

The IC50 values and slopes±S.E. or S.D. were determined by computer analysis of the graphical data illustrated in Figs. 1 and 2.

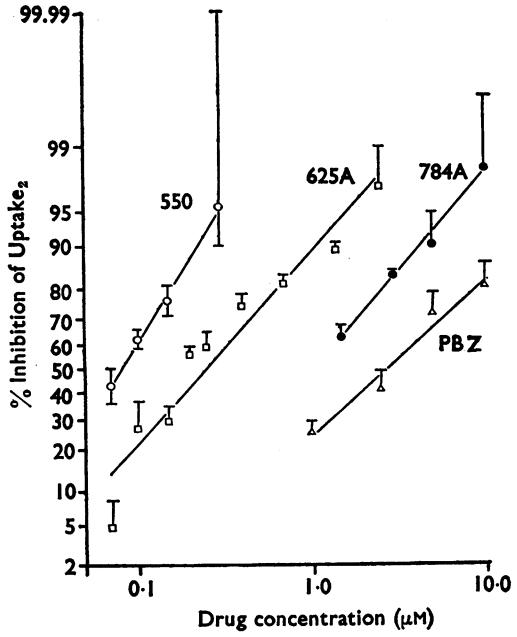


FIG. 1. Determination of Uptake$_2$ IC50 values for haloalkylamines in isolated rat heart. Percentage inhibition of ^{3}H-NA by Uptake$_2$ (probability scale) is plotted against log concentration of added inhibitor; lines were fitted by the method of least squares analysis. Each point is the mean value for six hearts; S.E.M. indicated by vertical lines. Compounds are indicated by their SKF numbers (see Tables 1 & 2), PBZ=phenoxybenzamine.

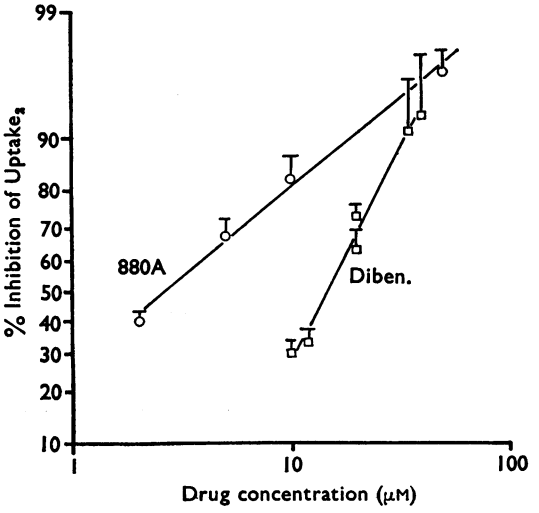


FIG. 2. Determination of Uptake$_2$ IC50 values for SKF 880A and dibenamine (Diben) in isolated rat heart, as described in Fig. 1. Note differences in slope of the log-probit plots.

to separate unchanged ^{3}H-NA from ^{3}H-normetanephrine and deaminated metabolites. In confirmation of previous studies (Lightman & Iversen, 1969), it was found that unchanged ^{3}H-NA accounted for more than 70% of the total radioactivity accumulated during a 4 min perfusion with 5 μg NA/ml (Table 5). The inhibitors reduced the accumulation of ^{3}H-NA by the same percentage as the reduction in total radioactivity. Measurements of ^{3}H-NA yielded IC50 values which were similar to those previously found (Table 5).

TABLE 5. *Effects of SKF 550 on extraneuronal uptake and metabolism of ^{3}H-noradrenaline in the isolated rat heart*

	Total uptake of ^{3}H-NA+metabolites μg/g	Uptake of unchanged ^{3}H-NA μg/g
Control	3·45±0·24 (0%)	2·70±0·32 (0%)
SKF 550—0·07 μM	1·96±0·31 (43·2%)	1·62±0·35 (40·1%)
SKF 550—0·10 μM	1·33±0·16 (62·3%)	1·08±0·62 (59·9%)
SKF 550—0·15 μM	0·83±0·22 (75·9%)	0·73±0·36 (73·0%)
SKF 550—0·30 μM	0·10±0·23 (95·4%)	0·07±0·17 (97·2%)

Effects of various concentrations of SKF 550 on total extraneuronal uptake of ^{3}H-NA+metabolites in isolated rat heart and on accumulation of unchanged ^{3}H-NA. Values are means±S.E.M. for 4–8 hearts, and are corrected for presence of extracellular ^{3}H-NA, and accumulation of labelled amine by neuronal uptake. Figures in parentheses indicate percentage inhibition of uptake by various concentrations of SKF 550.

Inhibition of Uptake$_1$

Some of the compounds described above were also tested as Uptake$_1$ inhibitors, using drug concentrations close to the Uptake$_2$ IC50 values previously determined (Table 6). The compounds SKF 550 and 625A caused no significant inhibition of Uptake$_1$ under these conditions, indicating that they are highly selective in their actions on Uptake$_2$. On the other hand, the compounds SKF 784A and dibenamine caused significant inhibition of Uptake$_1$ when tested at their Uptake$_2$ IC50 concentrations. The approximate values for Uptake$_1$ IC50 concentrations (Table 6) suggest that SKF 550 is approximately 500 times more potent as an Uptake$_2$

TABLE 6. *Inhibition of Uptake$_1$ by haloalkylamines*

Drug	Inhibition of Uptake$_1$ at concentration near IC50 for Uptake$_2$		Approximate IC50 for Uptake$_1$ μM
	Concentration μM	% Inhibition Uptake$_1$	
SKF 550	0·12	3·1±9·0	40·0 (Fig. 3)
SKF 625A	0·25	4·6±2·6	>50·0 (Fig. 4)
SKF 784A	2·00	34·5±9·6	4·5
Dibenamine	15·00	49·8±2·2	15·0
Phenoxybenzamine	—	—	0·9 (a)

(a) See Iversen & Langer (1969).

The inhibition of Uptake$_1$ in the isolated rat heart was measured during perfusion with various haloalkylamines at or near to their IC50 Uptake$_2$ concentrations previously determined. Each value is the mean±S.E.M. for 6 hearts. From these results, and those presented elsewhere (see references given) it was possible to estimate approximate IC50 values for Uptake$_1$ inhibition by these compounds.

inhibitor than as an Uptake$_1$ inhibitor; the corresponding ratio for SKF 625A is more than 400, for SKF 784A it is 2, for dibenamine 1 and for phenoxybenzamine 0·3.

The substance SKF 550 is thus not only the most potent inhibitor of Uptake$_2$, but also shows the greatest degree of selectivity in its actions on the two uptake processes. This compound inhibited Uptake$_1$ only at relatively high concentrations; this is illustrated by the results in Fig. 3, in which the inhibition of the total intracellular uptake of radioactivity in rat hearts is plotted against concentration of SKF 550. In this graph the values have not been corrected in the usual way for an Uptake$_1$ component in the total accumulation of radioactivity, and two

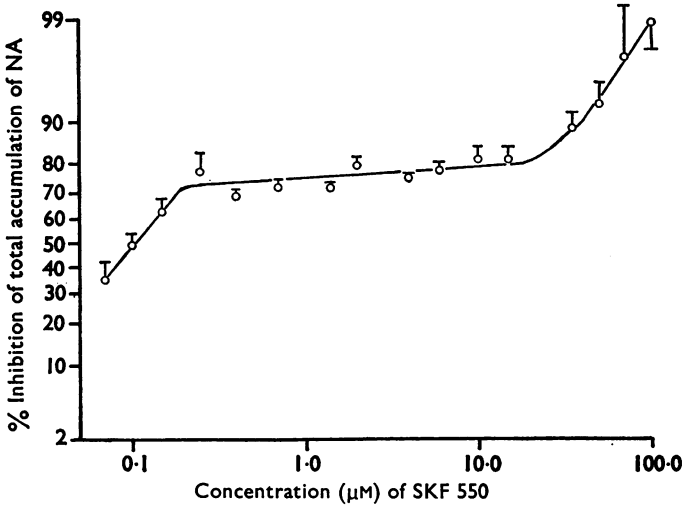


FIG. 3. Inhibition of accumulation of ^{3}H-NA in rat heart by SKF 550. Percentage inhibition of total intracellular ^{3}H-NA uptake (probability scale) is plotted against log concentration of inhibitor. Each point is the mean value for six hearts, S.E.M. indicated by vertical lines. These values, unlike those illustrated in Fig. 1, are not corrected for an $Uptake_1$ component in the total accumulation of radioactivity.

phases of inhibition are clearly evident. The first, at low concentrations of drug, corresponding to inhibition of $Uptake_2$, and the second, at much higher drug concentrations, to inhibition of the $Uptake_1$ component. The value of 20% of the total uptake attributable to $Uptake_1$ indicated by these results is in good agreement with the estimated $Uptake_1$ component which was routinely subtracted from other $Uptake_2$ results. A similar plateau effect for inhibition of $Uptake_2$ was observed with SKF 625A (Fig. 4), again confirming that the component resistant to inhibition (presumably due to $Uptake_1$) was approximately 20% of the total uptake. It must be pointed out, however, that for some of the compounds which were more

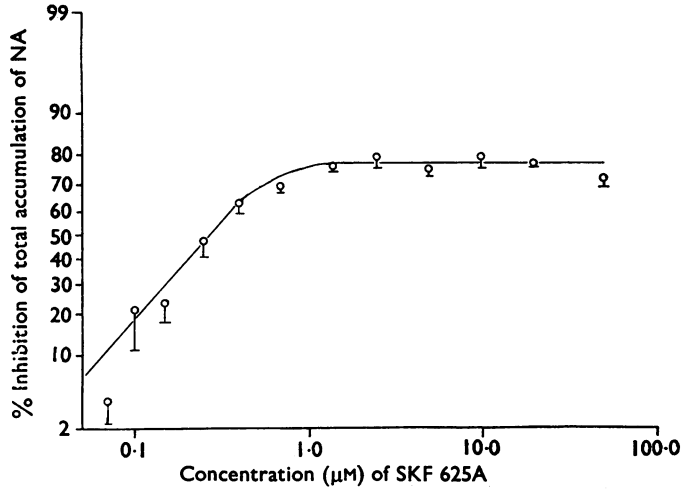


FIG. 4. Inhibition of total accumulation of ^{3}H-NA in rat heart by SKF 625A, as described in Fig. 3.

potent $Uptake_1$ inhibitors this correction is not entirely valid, so that some of the results given in Tables 1–3 may represent slight over-estimates of the inhibition of $Uptake_2$ produced by such compounds.

Discussion

In the present study, it has been shown that various structural analogues of phenoxybenzamine can act as inhibitors of the $Uptake_1$ and $Uptake_2$ mechanisms for catecholamine uptake in the rat heart. In studies of $Uptake_2$ inhibition, hearts were exposed briefly to a high perfusion concentration of ^{3}H-NA and the total intracellular accumulation of labelled NA and metabolites was used as a measure of $Uptake_2$. Since the extraneuronal metabolism of ^{3}H-NA appears to depend on the delivery of catecholamines to the metabolizing enzymes by $Uptake_2$ (Lightman & Iversen, 1969), this is believed to be a valid method for determining the extraneuronal uptake of catecholamine in this tissue. This is, furthermore, borne out by the finding that similar results were obtained for drug inhibition of $Uptake_2$ if the accumulation of unchanged ^{3}H-NA, rather than total radioactivity was measured. $Uptake_2$ measurements were also corrected for a predicted component of the total accumulation of radioactivity due to the neuronal uptake of catecholamine by the $Uptake_1$ mechanism. For compounds with only weak activity as $Uptake_1$ inhibitors this is a valid correction as illustrated in Figs. 4 and 5, although for substances with potent inhibitory effects on $Uptake_1$ this method may have led to slight over-estimates of the inhibitory effects on $Uptake_2$. Since the $Uptake_1$ component represents only 20% of the total accumulation of radioactivity, however, this error is unlikely to be substantial; furthermore, the high concentration of NA used for $Uptake_2$ experiments may compete with inhibitors at $Uptake_1$ sites, thus reducing their effect on this process.

The results obtained in the present study indicate that several haloalkylamines are both more potent and more selective inhibitors of $Uptake_2$ than phenoxybenzamine. The substances SKF 550 and 625A are of particular interest, since they are significantly more potent inhibitors of $Uptake_2$ than phenoxybenzamine and also have much less inhibitory activity than phenoxybenzamine on $Uptake_1$. These substances may represent valuable new tools for future investigations of the physiological and pharmacological importance of the $Uptake_2$ system. SKF 550, which is 35 times more potent an inhibitor of $Uptake_2$ than phenoxybenzamine but more than 40 times less potent on $Uptake_1$, has an α-adrenoceptor blocking activity, determined from antagonism of the pressor effects of adrenaline in the cat, equivalent to one-half or one-quarter that of phenoxybenzamine (Dr. G. Wilfon, personal communication). SKF 625A is approximately equipotent with phenoxybenzamine as an α-receptor blocking agent (Dr. W. Wilfon, personal communication) and is again considerably more potent than phenoxybenzamine on $Uptake_2$ and less potent on $Uptake_1$. It is apparent from these results, and from other similar comparisons within the series of compounds tested, that there is no direct correlation between the potency of haloalkylamines as α-adrenoceptor blocking agents and their actions as $Uptake_2$ or $Uptake_1$ inhibitors. Kalsner & Nickerson (1969) have similarly reported that the haloalkylamine derivative *N*-cyclohexylmethyl-*N*-ethyl-β-chloroethylamine (GD131), which has only weak α-receptor blocking activity, inhibits the extraneuronal uptake and metabolism of catecholamines in rabbit aorta.

Our results are not sufficiently complete to allow a detailed description of the structure activity requirements for $Uptake_2$ and $Uptake_1$ inhibition in haloalkylamines. However, it is of interest that *O*-methylation of *N*-phenylethyl or phenoxyethyl substituents enhanced both the potency and selectivity of these substances as $Uptake_2$ inhibitors. This finding, together with the generally greater activity of phenylethyl or *N*-phenoxyethyl substituted compounds in inhibiting both $Uptake_1$ and $Uptake_2$, might suggest that the structural requirements among haloalkylamines are similar to those reported for phenylethylamines by Burgen & Iversen (1965). The compound SKF 550, however, does not appear to fit in this category, and the reasons for the high potency of this compound as an $Uptake_2$ inhibitor are not clear. *N*-fluorenyl substituents have been found to increase the α-adrenoceptor blocking activity of related haloalkylamines (Graham, 1962), perhaps because such substituents facilitate the formation of the ethyleneiminium ion (Nickerson & Gump, 1949).

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