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INHIBITION OF EPINEPHRINE AND METARAMINOL UPTAKE INTO ADRENAL MEDULLARY VESICLES BY ARALKYLAMINES AND ALKYLAMINES

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Abstract— Two amine uptake mechanisms appeared to operate in isolated adrenal medullary storage vesicles; one site had a high affinity for epinephrine ($K_m \simeq 30$ μM) and low capacity ($U_{max} \simeq 20$ nmoles/100 μg of endogenous catecholamines), while the other had a low affinity ($K_m \simeq 2$ mM) and a higher capacity ($U_{max} \simeq 130$ nmoles). The low affinity site was non-specific and did not display competitive inhibition by agents which affected the high affinity, stimulated transport system. The high affinity system was inhibited in a purely competitive fashion by a variety of indoleamines and phenethylamines, but the two classes of compounds displayed different structure–activity relationships. Substitution on the α-carbon decreased the abilities of indoleamines to inhibit stimulated epinephrine uptake, but enhanced activity of phenethylamines. Ring hydroxylation reduced, and methoxylation eliminated, the inhibitory activity of tryptamine, but the same substituents markedly enhanced the activity of phenethylamines. Studies of compounds with restricted side-chain conformation indicated that a condensed structure favored activity in indoleamines, while an extended chain enhanced inhibition by phenethylamines. Linear alkylamines of 5- or 6-carbon length were also able to inhibit active epinephrine uptake. None of the agents inhibited the non-stimulated uptake component of metaraminol, which uses primarily the low affinity system. These data suggest that while indoleamines and phenethylamines do compete with epinephrine for attachment to the high affinity transport site in the vesicle membrane, the point of interaction is probably solely at the locus which binds the amine nitrogen; the remainder of the two types of molecule probably bind to at least two different sites adjacent to the *N*-binding area.

Over a decade ago, independent reports by Kirshner [1] and Carlsson *et al.* [2] demonstrated that isolated adrenal medullary vesicles can incorporate catecholamines by a mechanism which is stimulated by ATP-Mg^{2+} and inhibited by reserpine. Subsequent studies from several laboratories support the view that the incorporation proceeds via a two-step process: first, there is active, carrier-mediated transport of the amine across the vesicle membrane, followed secondarily by binding of the amine in the intravesicular storage complex [3–5]. In addition, a second transport system has been identified which is not stimulated by ATP-Mg^{2+} and is not blocked by reserpine [6–8]; this mechanism operates at higher amine concentrations and does not display the same specificity [3,8]. While epinephrine is incorporated primarily by the stimulated mechanism, metaraminol appears to utilize primarily the non-stimulated system [3,6–8].

Initial studies by Carlsson *et al.* [9] indicated that serotonin was incorporated into the vesicles to a greater extent than were the natural catecholamine substrates. Subsequently, Slotkin and Kirshner [3] showed that although the ATP-Mg^{2+}-stimulated incorporation of serotonin was indeed larger, the intravesicular binding of serotonin was less stable than that of epinephrine, suggesting that the large incorporation of serotonin probably resulted from differences in affinity for membrane transport. In a similar fashion, purified storage vesicle membranes were found to bind serotonin to a greater extent than epinephrine [10, 11], supporting the hypothesis that the transport system is responsible for the difference in incorporation. The difference in affinity can also account for the potent, competitive inhibition of epinephrine uptake by β-carboline derivatives [11, 12], such as harmine and reserpine, which can be viewed as substituted tryptamine derivatives in which the side chain has been cyclized.

The ATP-Mg^{2+}-stimulated transport system thus would appear to tolerate a wide variety of structural changes in the amine which attaches to it. While a number of reports have appeared in which various phenethylamines and tryptamines have been used as competing agents [1–3, 11, 12], systematic study of structure–activity relationships is obviously required to demonstrate conclusively whether tryptamine derivatives, alkylamines and non-catechol phenethylamines in fact do interact at the same site in the vesicle membrane, whether the effect is specific to the stimulated system, and whether the kinetics of interaction are truly competitive.

METHODS

Sprague–Dawley rats (Zivic–Miller) weighing 250–350 g were killed by decapitation and the adrenals were cleaned of fat and connective tissue, pooled and homogenized in an all-glass apparatus containing 1·25 ml/gland of ice-cold sucrose-Tris (300 mM sucrose buffered at pH 7·4 with 25 mM Tris–sulfate, containing 0·01 mM iproniazid to inhibit monoamine oxidase irreversibly). The homogenate was centrifuged at 800 *g* for 10 min to remove debris, and the vesicle-containing supernatant was used for subsequent

uptake determinations. In general, each gland provided enough material for two to three individual uptake measurements.

One-half-ml aliquots of the vesicle-containing suspension were added to tubes with 5 mM ATP-Mg^{2+}, 5 μCi ^{3}H-epinephrine or ^{3}H-metaraminol, and varying concentrations of epinephrine, metaraminol and drugs. Sucrose–Tris was added to give a final volume of 1 ml, and tubes were incubated in a water bath at 30° for 30 min and shaken gently, while duplicate tubes were kept on ice. Uptake was stopped by the addition of 2 ml of ice-cold sucrose–Tris and the samples were centrifuged for 10 min at 26,000 *g*. The supernatant fractions were deproteinized with perchloric acid and analyzed for radioactivity and catecholamines [8, 13] to determine the specific activity of the labeling medium. The vesicular pellets were washed and recentrifuged twice with fresh sucrose–Tris and the final pellet was deproteinized and analyzed for endogenous catecholamines and radioactivity. The uptakes at 30° and 0° were calculated as described previously [8] and the temperature-dependent component was obtained by subtracting the 0° uptake from the 30° value. Because of day-to-day variations in the activities of the preparations, control uptakes were determined with each set of experiments. More detailed descriptions of the methodology have been published elsewhere [3, 8, 11].

In studies in which *N*-ethylmaleimide (NEM) was used, the vesicles were first pre-incubated with NEM alone for 5 min at 30°, and ATP-Mg^{2+}, amines and radioactivity were added subsequently; in those cases, controls were also pre-incubated, but without addition of NEM. With the exception of studies with NEM, there was little or no change in endogenous or exogenous catecholamines throughout the 30-min incubation.

In some experiments, the washed, labeled pellet was resuspended in fresh sucrose–Tris instead of perchloric acid, layered on continuous sucrose density gradients [3], and centrifuged at 105,000 *g* for 16 hr. In all cases, the distribution of labeled amines was identical to endogenous catecholamines, indicating that labeling occurred solely in the storage vesicles, despite the presence of other organelles in the 26,000 *g* pellet [3, 14, 15].

It is important to note that, as used here, the term "uptake" refers to a complex process involving inward transport, intravesicular binding and outward leakage of amines. While a system of this type is not adequately described by simple enzyme kinetics, mathematical analysis indicates that agents which act competitively at the membrane transport site will produce a change in K_m but no change in U_{max} (maximal uptake) [16]. On the other hand, drugs which are non-competitive inhibitors of transport or which compete at intravesicular binding sites will produce a kinetic picture analogous to classical non-competitive inhibition (no change in K_m but a reduction in U_{max} [16]. In the present study, several experiments were conducted utilizing a range of epinephrine concentrations in the presence and absence of drugs to enable determination of the type of inhibition; these data are presented as Lineweaver–Burk plots [17]. Again, because of day-to-day variations in activities, control curves were determined with each preparation.

Data are reported as means and standard errors, and levels of significance calculated by Student's *t*-test [18]. Straight lines are determined by the method of least squares [18].

d,l-Epinephrine-7-^{3}H and *d,l*-metaraminol-7-^{3}H were obtained from New England Nuclear Corp. *l*-Epinephrine bitartrate was obtained from Winthrop Laboratories and *l*-metaraminol bitartrate from Merck, Sharp & Dohme. Phenethylamine, indoleamine and alkylamine derivatives were purchased from Aldrich Chemical Co. and reserpine phosphate from Ciba Pharmaceuticals.

RESULTS

The uptake of epinephrine into adrenal storage vesicles displayed a biphasic dependence on concentration (Fig. 1): at concentrations below 0·1 mM, the K_m for uptake was approximately 30 μM and the maximal uptake (U_{max}) was 24 nmoles/100 μg of endogenous catecholamines. At concentrations above 0·8 mM, uptake occurred via a second system with lower affinity and higher capacity (K_m = 2 mM, U_{max} = 130 nmoles/100 μg of endogenous catecholamines). Therefore, in subsequent experiments at single epinephrine concentrations, 0·1 mM was chosen because: (1) a fairly high concentration obviates sample-to-sample variations in external amine concentration which might result from small degrees of lysis and leakage of vesicles; (2) the high affinity site is approximately 80 per cent saturated and the measured uptake is close to maximal; and (3) the concentration is sufficiently low such that the contribution of the low affinity system to the observed uptake is small ($\lesssim$ 20 per cent).

The abilities of indoleamines to inhibit epinephrine uptake are shown in Table 1. In concentrations equimolar with epinephrine, tryptamine produced nearly 80 per cent inhibition, while the 5- and 6-hydroxylated derivatives were only slightly less effective. On the other hand, substitution of methoxyl or methyl groups at the 5-position markedly reduced activity.

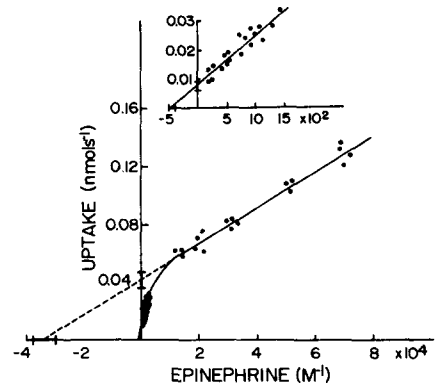

Fig. 1. Lineweaver–Burk plot of epinephrine uptake into isolated rat adrenal medullary storage vesicles, showing the contributions of two uptake components. For the high affinity site, K_m = 31·8 ± 1·9 μM and U_{max} = 23·8 ± 1·4 nmoles/100 μg of endogenous catecholamines. Inset shows expanded view of high concentrations data; for the low affinity site, K_m = 2·16 ± 0·27 mM and U_{max} = 129 ± 16 nmoles/100 μg of endogenous catecholamines.

Table 1. Effects of indoleamines on uptake of ^{3}H-epinephrine into isolated rat adrenal medullary storage vesicles.*

Drug (0·1 mM)	Percentage inhibition of epinephrine uptake	No. of determinations
Control	0 ± 5	18
Tryptamine	78 ± 2	18
5-Hydroxytryptamine (serotonin)	56 ± 2	6
5-Methoxytryptamine	18 ± 3	6
5-Methyltryptamine	27 ± 3	6
6-Hydroxytryptamine	62 ± 2	6
α-Methyltryptamine	26 ± 4	6
α-Ethyltryptamine	36 ± 2	6
N-methyltryptamine	75 ± 2	6
Homotryptamine	79 ± 4	6
Gramine	9 ± 4	6
Harmine	85 ± 1	6

* Epinephrine concentration in the medium was 0·1 mM. Control epinephrine uptake was 19·9 ± 0·9 nmoles/100 μg of endogenous catecholamines.

Alterations in the side chain also influenced the inhibition; α-substitution resulted in partial loss of activity, but *N*-substitution did not. Lengthening the side chain by one carbon (homotryptamine) left inhibitory activity intact, but shortening the chain by one carbon (gramine, also *N*-substituted) produced an inactive compound. Condensation of the side chain to the indole nucleus (harmine) led to a high degree of inhibition.

To determine the specificity of the inhibition of epinephrine uptake by indoleamines, the effects of several derivatives on metaraminol uptake were measured (Table 2). Tryptamine, 5- and 6-hydroxytryptamine and harmine all produced a partial reduction in metaraminol uptake, but the magnitude of the inhibition was much smaller than for epinephrine uptake; 5-methoxytryptamine, which was a poor inhibitor of epinephrine uptake, had no effect on metaraminol uptake. While metaraminol uses primarily the non-stimulated, low affinity system for uptake, as much as 40 per cent of the uptake may result from the stimulated system, if the external epinephrine concentration is low [3, 8]. When 0·1 mM epinephrine was added to the incubation mixture (to saturate the stimulated mechanism), the uptake of metaraminol was reduced by about 30 per cent and the inhibitory activities of the derivatives were virtually eliminated (Table 2), indicating specificity of the indoleamines for the high affinity system.

Similar studies were conducted to evaluate the influence of various substituent groups on the inhibition by phenethylamines (Table 3); in general, higher concentrations of these inhibitors were required than was the case with indoleamines. β-Phenethylamine at 0·3 mM produced only a 25 per cent inhibition of epinephrine uptake into rat adrenal vesicles. This is in sharp contrast to the effectiveness of β-phenethylamine in cow adrenal vesicles [3, 11], indicating a species dependence of the uptake system. Addition of a *para*-hydroxyl group (tyramine) markedly enhanced inhibitory activity, but addition of the second ring hydroxyl (dopamine) did not produce a further increment in inhibition. Surprisingly, norepinephrine (addition of β-hydroxyl) was quite ineffective, while subsequent *N*-substitution to form epinephrine or isoproterenol restored the affinity for the transport system; this also contrasts with cow vesicles, where little distinction is made between epinephrine and nore-

Table 2. Effects of indoleamines on uptake of ^{3}H-metaraminol into isolated rat adrenal medullary storage vesicles.*

Drug (0·1 mM)	Percentage inhibition of metaraminol uptake		No. of determinations
	No epinephrine added	Epinephrine added (0·1 mM)	
Control	0 ± 3	0 ± 4	6;6
Tryptamine	25 ± 2	10 ± 2	6;6
5-Hydroxytryptamine	30 ± 1	10 ± 2	6;6
5-Methoxytryptamine	2 ± 2	−4 ± 2	6;6
6-Hydroxytryptamine	34 ± 2	11 ± 3	6;6
Harmine	43 ± 2	12 ± 4	6;6

* Epinephrine concentration in the medium ranged from 5 to 7 μM in samples without added epinephrine. Metaraminol concentration in the medium was 0·1 mM throughout. Control metaraminol uptakes were 4·83 ± 0·14 nmoles/100 μg of endogenous catecholamines (no epinephrine added) and 3·39 ± 0·15 nmoles/100 μg of endogenous catecholamines (0·1 mM epinephrine added).

Table 3. Effects of phenethylamines on uptake of ^{3}H-epinephrine into isolated rat adrenal medullary storage vesicles: alterations in substituent groups*

Drug	Percentage inhibition of epinephrine uptake	No. of determinations
Concentration (0·3 mM):		
Control	0 ± 6	25
β-Phenethylamine	26 ± 2	4
Tyramine	51 ± 3	4
Dopamine	51 ± 2	21
Norepinephrine	26 ± 2	16
Epinephrine	60 ± 3	4
Isoproterenol	54 ± 2	4
Salsolinol	39 ± 2	12
Apomorphine	69 ± 3	5
Concentration (1 mM):		
Control	0 ± 5	6
β-Phenethylamine	36 ± 3	6
Amphetamine	48 ± 2	6
Tyramine	73 ± 3	6
p-Cl-phenethylamine	91 ± 3	6
Norepinephrine	61 ± 1	6
Normetanephrine	56 ± 2	6
3,4-Dimethoxyphenethylamine	85 ± 3	6

* Epinephrine concentration in the medium was 0·1 mM. Control epinephrine uptakes were: 16·1 ± 0·9 nmoles/100 μg of endogenous catecholamines (first set), and 21·2 ± 1·1 nmoles/100 μg of endogenous catecholamines (second set).

pinephrine [1, 3]. Condensation of the side chain to the phenyl ring reduced activity somewhat (salsolinol), while extensive substitutions which freeze the chain in an extended conformation (apomorphine) actually enhanced inhibition. Earlier studies [16] have shown that metaraminol is ineffective in inhibiting epinephrine uptake.

In contrast to indoleamines, α-substitution (amphetamine) enhanced the inhibitory effect of β-phenethylamine (Table 3) and the replacement of ring hydroxyls with methoxyl groups not only did not reduce activity (normetanephrine), but may actually increase the inhibition (3,4-dimethoxyphenethylamine). Replacement of the *para*-hydroxyl group with a more electronegative substituent (*p*-chlorophenethylamine) produced a powerful inhibitory agent.

Lengthening the side chain of phenethylamine by one or two carbon atoms enhanced the inhibition of epinephrine uptake, while shortening by one or more carbon atoms markedly reduced activity in a fashion similar to that seen with indoleamines (Table 4). When the side chain was eliminated, *para*-hydroxylation no longer enhanced inhibitory activity (*p*-aminophenol).

To determine whether an aromatic nucleus was required for activity, the inhibition of epinephrine uptake by straight chain alkylamines was studied. Ethylamine, propylamine and butylamine were ineffective, but 5- and 6-carbon amines were approximately as effective as the phenethylamine-phenbutylamine series (Table 5).

Table 4. Effects of phenalkylamines on uptake of ^{3}H-epinephrine into isolated rat adrenal medullary storage vesicles: alterations in length of side chain*

Drug	Percentage inhibition of epinephrine uptake	No. of determinations
Concentration (1 mM):		
Control	0 ± 10	7
β-Phenethylamine	31 ± 4	8
γ-Phenpropylamine	48 ± 4	8
δ-Phenbutylamine	64 ± 8	8
Concentration (3 mM):		
Control	0 ± 6	4
δ-Phenbutylamine	79 ± 5	4
γ-Phenpropylamine	75 ± 4	4
β-Phenethylamine	66 ± 2	4
Benzylamine	22 ± 3	4
Aniline	29 ± 4	4
Pyridine	16 ± 2	4
p-Aminophenol	7 ± 3	4

* Epinephrine concentration in the medium was 0·1 mM. Control epinephrine uptakes were: 18·0 ± 1·8 nmoles/100 μg of endogenous catecholamines (first set), and 20·5 ± 1·3 nmoles/100 μg of endogenous catecholamines (second set).

Table 5. Effects of alkylamines on uptake of ^{3}H-epinephrine into isolated rat adrenal medullary storage vesicles*

Drug (3 mM)	Percentage inhibition of epinephrine uptake	No. of determinations
Control	0 ± 6	8
Ethylamine	−5 ± 3	4
n-Propylamine	−11 ± 6	4
n-Butylamine	−15 ± 15	4
n-Amylamine	48 ± 3	4
n-Hexylamine	73 ± 3	4

* Epinephrine concentration in the medium was 0·1 mM. Control epinephrine uptake was 14·9 ± 0·9 nmoles/100 μg of endogenous catecholamines.

The effects of phenethylamine derivatives on metaraminol uptake were similar to those of the indoleamines: the inhibition seen with no added epinephrine was eliminated when epinephrine was added to saturate the high affinity system (Table 6), indicating specificity of the inhibitors for the ATP-Mg^{2+}-stimulated uptake.

To demonstrate conclusively whether these agents act at the same carrier site which transports epinephrine, the kinetics of inhibition of the high affinity system were evaluated for several derivatives. A typical experiment for tryptamine appears in Fig. 2. The inhibition of epinephrine uptake by tryptamine displayed purely competitive kinetics, and the K_i for tryptamine was lower than the K_m for epinephrine, indicating a higher affinity of the indoleamine for the system. The results of other kinetic determinations are summarized in Table 7. Harmine, reserpine, β-phenethylamine, *p*-chlorophenethylamine and hexylamine all were competitive with epinephrine, although the values of K_i spanned several orders of magnitude. The results for reserpine agree closely with those of Jonasson *et al.* [19] for cow vesicles. In contrast, *N*-ethylmaleimide, a relatively non-specific sulfhydryl-reactive agent, produced a non-competitive inhibition of epinephrine uptake. The high values for U_{max} seen in the latter experiment result from the partial depletion of endogenous catecholamines during the 5-min pre-incubation in the absence of ATP-Mg^{2+} [20]; the reduction in catecholamines/vesicle increases the magnitude of uptake/100 μg of endogenous catecholamines.

DISCUSSION

The uptake of metaraminol into adrenal medullary vesicles had been shown previously to differ in several respects from that of catecholamines [3, 6–8]; these included lack of stimulation by ATP-Mg^{2+} and inability of reserpine to inhibit uptake. In the present study, it has been demonstrated that epinephrine, in high concentrations, can also utilize the non-stimulated system. The values obtained for K_m and U_{max} agree closely with those reported for metaraminol [16], indicating that the system does not display the type of specificity associated with the high-affinity, stimulated system. Furthermore, none of the agents tested here appeared to inhibit the non-stimulated system. The saturation kinetics indicate, however, that this is not simple diffusion, but more likely a passive, carrier-mediated, "downhill" transport. The high K_m, lack of specificity and inability to elicit competition, and lack of requirement for energy all suggest that uptake which proceeds via this mechanism is probably non-physiological; for example, a mobile membrane protein which has non-specific, amine-binding sites, but whose primary function is not transport,

Table 6. Effects of phenethylamines on uptake of ^{3}H-metaraminol into isolated rat adrenal storage vesicles*

Drug (1 mM)	Percentage inhibition of metaraminol uptake		No. of determinations
	No epinephrine added	Epinephrine added (0·1 mM)	
Control	0 ± 11	0 ± 13	6;6
β-Phenethylamine	35 ± 6	4 ± 8	6;6
p-Cl-phenethylamine	57 ± 9	−13 ± 11	6;6
3,4-Dimethoxyphenethylamine	43 ± 2	1 ± 7	6;6

* Epinephrine concentration in the medium ranged from 6 to 8 μM in samples without added epinephrine. Metaraminol concentration in the medium was 0·1 mM throughout. Control metaraminol uptakes were: 8·20 ± 0·89 nmoles/100 μg of endogenous catecholamines (no epinephrine added) and 4·43 ± 0·56 nmoles/100 μg of endogenous catecholamines (0·1 mM epinephrine added).

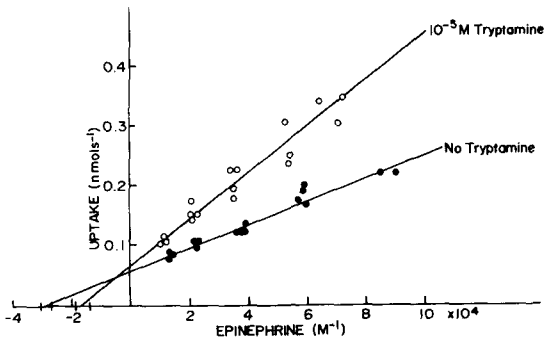

Fig. 2. Lineweaver–Burk plot of epinephrine uptake into isolated rat adrenal medullary storage vesicles in the presence (○) and absence (●) of 10^{-5} M tryptamine. Intercepts on the ordinate are not significantly different, while intercepts on the abscissa are ($P < 0.02$).

could be made to carry amines passively to the interior of the vesicle provided that the amine concentration on the outside is sufficiently higher than on the inside. However, the non-stimulated mechanism may be of pharmacological importance in that it enables false transmitters (like metaraminol) to enter the vesicle even if they are poor utilizers of the stimulated system.

The structure-activity relationships of indoleamines, and of phenethylamines for the stimulated system demonstrated both similarities and disparities. In both series, *N*-substitution did not reduce the inhibitory activity and, in both, at least two or more carbons were required in the side chain. However, while α-substitution markedly decreased the activity of indoleamines, it enhanced that of phenethylamines, and while for indoleamines, condensation of the side chain with the ring system enhanced activity (harmine), the same condensation for phenethylamines (salsolinol) decreased activity; furthermore, restriction of the phenethylamine side chain to an extended conformation (apomorphine) enhanced activity. These data imply that although both types of compounds attach to the *N*-binding site of the transport molecule, the side chains of indoleamines and phenethylamines do not occupy the same locus. In addition, there was a complete lack of correlation in the effects of ring substituents in indoleamines vs phenethylamines (Tables 1 and 3), suggesting that the two aromatic nuclei occupy different areas.

Despite these differences, both types of inhibitors displayed purely competitive kinetics, indicating that they do in fact attach to part of the site to which epinephrine binds. The picture that emerges is that of a single binding site for the amino nitrogen with at least two available channels for side chains, each with different binding areas for the rings and ring substituents. One area may be simply hydrophobic, since long-chain alkylamines can act as inhibitors. The concept of several auxiliary binding areas has been postulated to explain the actions of reserpine on vesicular uptake [11] and in receptor systems to describe the activities of antimuscarinic agents [21].

Several differences were noted in the specificity of the stimulated uptake system in rat vesicles as compared to those previously reported in cows [1–3, 8], and there was also a disparity in the K_m obtained in the two species [19], although the latter difference may be methodological in origin [11, 16]. These data indicate that caution should be exercised in extrapolation of results from one species to another. The marked preference for epinephrine vs norepinephrine in the rat may be of functional significance, since 70–80 per cent of the stored catecholamines in the rat adrenal medulla consists of epinephrine.

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Table 7. Kinetic constants for ^{3}H-epinephrine uptake into isolated rat adrenal medullary storage vesicles*

Drug	K_m for epinephrine (μM)	U_{max} for epinephrine (nmoles/100 μg of endogenous catecholamines)	No. of uptake points used in determination	Type of inhibition	K_i for inhibitor (μM)
Control	34·1 ± 3·0	17·7 ± 1·6	17		
Tryptamine (10 μM)	56·9 ± 8·8†	14·7 ± 2·3	19	Competitive	15·0 ± 2·3
Harmine (10 μM)	156 ± 6‡	16·8 ± 1·2	19	Competitive	2·80 ± 0·10
Control	64·8 ± 4·8	21·0 ± 1·5	68		
Reserpine (25 nM)	109 ± 17§	21·1 ± 3·3	67	Competitive	0·037 ± 0·006
Control	27·0 ± 2·1	17·2 ± 1·3	19		
β-Phenethylamine (1 mM)	84·0 ± 18·4‖	13·0 ± 2·8	19	Competitive	474 ± 104
Control	56·8 ± 3·3	30·6 ± 1·8	25		
p-Cl-phenethylamine (0·1 mM)	208 ± 65†	21·4 ± 6·9	25	Competitive	37·5 ± 11·7
Control	34·0 ± 1·5	17·4 ± 0·8	25		
n-Hexylamine (1 mM)	256 ± 56‡	30·1 ± 6·5	24	Competitive	153 ± 33
Control	44·6 ± 3·2	40·3 ± 2·9	25		
N-ethylmaleimide (50 μM)	42·6 ± 2·3	29·7 ± 1·6‖	25	Non-competitive	99·5 ± 5·4

* Epinephrine concentrations in the medium ranged from 10 to 80 μM. Kinetic parameters are calculated from least squares determinations of Lineweaver–Burk plots. K_i (competitive) is calculated from the equation $K_i = K_m I/(K_{m'} - K_m)$, where K_i is the Michaelis constant for the inhibitor, K_m is the Michaelis constant for epinephrine, $K_{m'}$ is the Michaelis constant for epinephrine in the presence of inhibitor and I is the inhibitor concentration. For non-competitive inhibition, $K_i = U_{max'} I/(U_{max} - U_{max'})$, where $U_{max'}$ and U_{max} are maximal uptake in the presence and absence of inhibitor respectively.

†$P < 0.02$ vs control.

‡$P < 0.001$.

§$P < 0.01$.

‖$P < 0.005$.

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