Naunyn-Schmiedeberg's Arch Pharmacol (1982) 319:66–70

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Inhibition of in vitro and ex vivo Uptake of Noradrenaline and 5-Hydroxytryptamine by Five Antidepressants; Correlation with Reduction of Spontaneous Firing Rate of Central Monoaminergic Neurones

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Summary. The principal neurochemical property of tricyclic antidepressants is the blockade of noradrenaline (NA) and/or 5-hydroxytryptamine (5-HT) uptake into monoaminergic nerve endings. Electrophysiological studies show that these drugs also decrease the firing rate of the noradrenergic neurones of the locus coeruleus (L.C.) and of the serotonergic neurones of the dorsal raphe (D.R.). In order to assess the relation between the two phenomena, the influence of five tricyclic antidepressants on NA and 5-HT uptake was studied in vitro. The concentrations required to produce a 50% inhibition (IC_{50}) were determined and correlated with the respective doses required to reduce to 50% (ID_{50}) the firing rate of L.C. and D.R. neurones. Ex vivo experiments were also performed to study the influence of the tricyclic antidepressants on NA and 5-HT uptake when administered i.v. at the doses decreasing to 50% the firing rate of L.C. and D.R. cells.

The inhibition of the NA uptake by tricyclic antidepressants can account, at least in part, for the inhibition of the firing rate of L.C. neurones observed after acute i.v. administration. In the case of serotonergic neurons, the results do not allow a firm conclusion.

Key words: Noradrenaline and 5-hydroxytryptamine uptake – Tricyclic antidepressants – Locus coeruleus – Dorsal raphe

Introduction

In vitro and ex vivo uptake studies have shown that tricyclic antidepressant drugs are potent inhibitors of the uptake of NA and of 5-HT by nerve endings in the peripheral as well as in the central nervous system (Hertting et al. 1961; Dengler et al. 1961; Axelrod et al. 1962; Glowinski and Axelrod 1964; Ross and Renyi 1967a, b). Tricyclic antidepressants with a secondary amine (desipramine, nortriptyline) are more potent inhibitors of NA uptake than their tertiary amine analogues (imipramine, clomipramine and amitriptyline), which in turn are more potent blockers of 5-HT uptake (Carlsson et al. 1969a, b; Carlsson 1970; Shaskan and Snyder 1970; Hamberger and Tuck 1973; Ross and Renyi 1975a, b).

Electrophysiological studies have shown that the systemic administration of these drugs reduces the firing rate of central noradrenergic and serotonergic neurones (Sheard et al. 1972; Nybäck et al. 1975). In a previous work, we made a quantitative comparison of the effect of these various tricyclic antidepressants on the firing rate of L.C. noradrenergic cells and of D.R. serotonergic cells (Scuvée-Moreau and Dresse 1979). In this work, the i.v. injection of low doses of antidepressants induced a progressive decrease in the frequency of discharge of the cells. The total doses necessary to produce a 50% reduction of the firing rate (ID_{50}) were determined. These experiments demonstrated great differences in potency and in selectivity between the drugs tested.

As the decrease in the firing rate could be a compensatory effect related to the increased availability of neurotransmitter in the synaptic cleft, the present work was initiated to obtain data for comparing the efficacy of the tricyclic antidepressants as blockers of NA or 5-HT uptake in vitro with their efficacy in decreasing the firing rate of L.C. or D.R. neurones. We have also determined ex vivo, the percent inhibition of NA and 5-HT uptake caused by the injection of the tricyclic antidepressants at the doses required to reduce to 50% the firing rate of L.C. and D.R. neurones.

Methods

In vitro Uptake Experiments. Female Wistar rats weighing 200–300 g were used in all experiments. The animals were sacrificed by decapitation, their brain quickly removed and the cerebral cortex sliced on ice. The brain cortex slices (0.1 mm thick) were pooled and 8 portions of about 30–50 mg were placed into various tubes. In each experiment, 4 tubes contained only Krebs-Henseleit buffer (control) and 4 tubes contained the medium with the uptake inhibitor. These tubes were preincubated 5 min in an atmosphere of 95% O_2 – 5% CO_2 at 37°C. ^{3}H-5-HT (0.2 nmole) and ^{14}C-NA (0.2 nmole) were added to produce a final concentration of 10^{-7} mol/l of each amine and the incubation was continued during 5 min. The reaction was stopped by chilling the tubes in ice cold water. 0.1 ml of the supernatant fluid was taken and added to a scintillation vial with 10 ml of Dimilume 30 Packard. The medium was decanted, the slices filtrated and washed with 20 ml ice cold Krebs-Henseleit solution. The slices were disintegrated in a counting vial with 1 ml Soluene 100 Packard and 10 ml Dimilume 30 scintillator were added to the vials. Radioactivity of the tissues and media was counted by a liquid scintillation spectrometer. The double labelling technique was used for determining NA and 5-HT uptake simultaneously. This allows a direct comparison of the inhibitory effects of a compound on the 2 uptake mechanisms. In order to study the uptake in optimal conditions,

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0028-1298/82/0319/0066/$01.00

several precautions were taken: 1) the amount of cortical brain slices was chosen in the range where the uptake was proportional to the amount of tissue per assay, 2) a concentration of 10^{-7} mol/l of amines was used in the incubation medium in order to limit as much as possible non specific processes (Shaskan and Snyder 1970), 3) a short incubation time has been used to limit the importance of metabolic transformations so that total radioactivity could be taken as a measure of amine uptake.

Tissue/medium ratios (T/M) of ^{3}H-5-HT and ^{14}C-NA were calculated as dpm per gram wet weight of tissue to dpm per ml of medium. The percent inhibition of amine uptake was calculated according to the following formula:

$$\frac{Rc - Ri}{Rc} \times 100$$

where *Ri* is the T/M ratio evaluated after incubation of cortical brain slices in the presence of an uptake inhibitor and *Rc* the ratio calculated after incubation of the slices in Krebs-Henseleit buffer only (control). The IC_{50} values were calculated mathematically from a linear regression applied to the points obtained by plotting the percent inhibitions of uptake against the corresponding concentrations of inhibitor.

In the kinetic studies, slices of cerebral cortex were incubated as described above with ^{3}H-5-HT and ^{14}C-NA concentrations ranging from 0.02 mol/l ^{3}H-5-HT to 3.5 mol/l. Ten tubes at least were incubated for each concentration. The velocity of amine uptake was expressed in nanomoles of amines accumulated per gram of wet weight of tissue per 5 min.

Ex vivo Uptake Experiments. These experiments were performed on female Wistar rats weighing 200 – 300 g. The animals were anaesthetized with chloral hydrate (400 mg kg^{-1} i.p.) and a catheter was placed in their jugular vein making possible the injection of the drugs under the same conditions as used in the previous electrophysiological studies (Scuvée-Moreau and Dresse 1979). Six animals were injected in each experiment: 3 of them received the antidepressant studied, 3 of them received saline. Several experiments were performed with the various drugs studied at a fixed dose. The animals were sacrificed by decapitation immediately after the perfusion and the uptake studies were run as described above. The percent inhibition of uptake was calculated according to the previous formula where *Ri* is now the T/M ratio evaluated after incubation of cortical brain slices of the rats perfused with the drug and *Rc* the ratio calculated after simultaneous incubation of cortical brain slices from the control rats.

Statistics. For statistical analysis of the results Student's *t*-test or Scheffe's multiple comparisons test were used. A P value < 0.05 was considered significant.

Compounds. The drugs used in this study were: desipramine HCl, imipramine HCl and clomipramine HCl (Ciba-Geigy, Basel, Switzerland), nortriptyline HCl (Eli Lilly, Indianapolis, IN, USA) and amitriptyline HCl (Merck, Sharp & Dohme, West Point, PA, USA). These drugs were dissolved in NaCl 0.9%. Doses refer to the bases. ^{3}H-5-Hydroxytryptamine creatinine sulfate and 1-(methylene-^{14}C)noradrenaline d-bitartrate were purchased from the Radiochemical Center, Amersham, Great Britain. Their specific activities were 10.7 curies/mmole for ^{3}H-5-HT and 57 mcuries/mmole for ^{14}C-NA.

Results

Inhibition of NA and 5-HT Uptake by Tricyclic Antidepressants in vitro

Correlation with Electrophysiological Studies. The IC_{50} values determined for the various tricyclic antidepressants are presented in Fig. 1 A and B. The order of potency of the tricyclic compounds to inhibit NA uptake is desipramine > nortriptyline > imipramine > amitriptyline > clomipramine. A comparison with previous electrophysiological data presented in Fig. 1 A shows that the order of potency of tricyclic antidepressants to inhibit the firing rate of L.C. noradrenergic neurones is the same as their order of potency to block NA uptake.

The order of potency of the drugs to inhibit 5-HT uptake is clomipramine > imipramine > amitriptyline > nortriptyline > desipramine (Fig. 1 B). A comparison with previous electrophysiological data (Fig. 1 B) shows that these compounds exhibit the same order of activity as inhibitors of the firing rate of D.R. serotonergic neurones.

A regression analysis applied to the points obtained by plotting electrophysiological data against uptake data gives a coefficient of linear correlation equal to $r = 0.9582$ ($P < 0.01$) in the case of NA and equal to $r = 0.9958$ ($P < 0.001$) in the case of 5-HT.

Inhibition of NA and 5-HT Uptake Caused by the i.v. Injection of the Tricyclic Antidepressants at the Doses Required to Produce a 50% Reduction of the Firing Rate of L.C. and D.R. Neurones

The five tricyclic antidepressants injected at the respective doses required to induce a 50% decrease in the firing rate of L.C. neurones produce a significant (Student's *t*-test) inhibition of NA uptake into cortical brain slices. The mean percent inhibitions of NA uptake are presented in Table 1. Statistical analysis of the whole results with Scheffe's test shows that the percent inhibition calculated for imipramine is different ($P < 0.05$) from the percent inhibition obtained with desipramine and nortriptyline. There is no statistically significant difference between the other results.

The five tricyclic antidepressants injected at the respective doses required to produce a 50% decrease in the firing rate of D.R. neurones lead only to a weak inhibition of 5-HT uptake into cortical brain slices. These results are presented in Table 1. In most of these experiments, there is no statistically significant difference (Student's *t*-test) between 5-HT uptake into cortical brain slices from rats injected with a tricyclic antidepressant and 5-HT uptake into cortical slices from control rats injected with saline.

Kinetics of NA and 5-HT Uptake in Cortical Brain Slices

Kinetic analysis performed by Shaskan and Snyder (1970) in the striatum and hypothalamus indicate two components of 5-HT accumulation representing a high and a low affinity transport system. These authors conclude that 5-HT probably accumulates not only in serotonergic neurones but also in catecholaminergic neurones. As this nonspecificity could perhaps explain the weak inhibition of 5-HT uptake ex vivo (Table 1), it seemed interesting to us to perform kinetic analysis in the cortical brain region in order to assess the specificity of NA and 5-HT uptake in our experimental conditions.

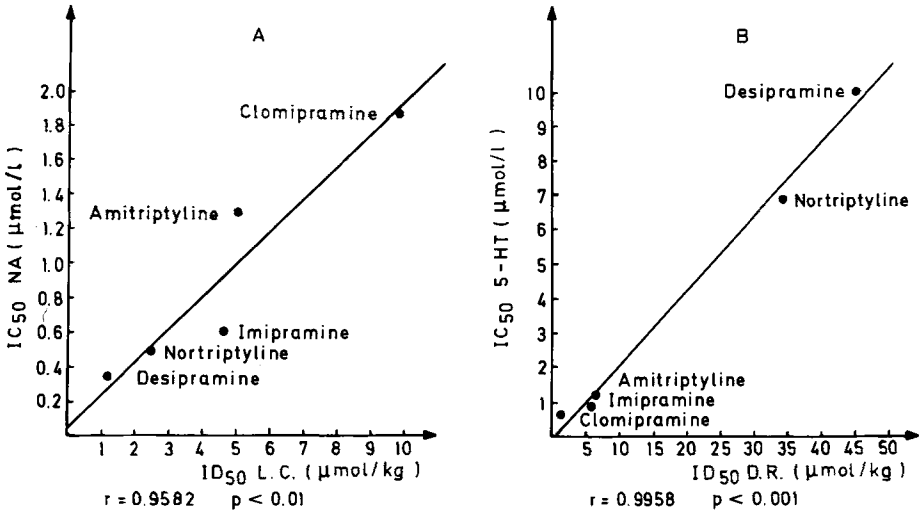

Fig. 1A and B
Correlation between inhibition of uptake of NA and 5-HT in vitro (this work) and inhibition of the firing rate of L.C. and D.R. neurones by various tricyclic antidepressants (from Scuvée-Moreau and Dresse 1979). (**A**) Relation between the concentrations necessary to induce a 50% inhibition of NA uptake (IC_{50}) and the doses required to produce a 50% decrease of the firing rate of L.C. neurones (ID_{50}). (**B**) Relation between the concentrations necessary to induce a 50% inhibition of 5-HT uptake (IC_{50}) and the doses required to produce a 50% reduction of the frequency of discharge of D.R. neurones (ID_{50})

Table 1. Percent inhibitions of NA and 5-HT uptake measured in cerebral cortex slices of rats injected with various antidepressant drugs at the doses which reduce to 50% (ID_{50}) the firing rate of L.C. noradrenergic neurones or D.R. serotonergic neurones. n = number of experiments. Statistical analysis: Student's t-test (comparison of the results obtained with the rats injected with the drugs and those obtained with the control rats injected with saline)

Antidepressant	ID_{50} (mg kg^{-1}) for depression of firing rate[b]		Percent inhibitions of uptake (mean ± S.E.)		n
	L.C.	D.R.	^{14}C-NA	^{3}H-5-HT	
Desipramine	0.3		39 ± 1**	3 ± 3[a]	9
		12	73 ± 1**	11 ± 1[a]	5
Imipramine	1.3		50 ± 1**		6
		1.6		20 ± 2[a]	6
Clomipramine	3.0		43 ± 1**	51 ± 1*	6
		0.35	9 ± 1[a]	19 ± 1*	6
Nortriptyline	0.66		34 ± 2**	3 ± 2[a]	6
		9	72 ± 2**	16 ± 4[a]	6
Amitriptyline	1.4		41 ± 2**		6
		1.8		15 ± 1[a]	6

[a] Not significant, $*P < 0.05$, $**P < 0.005$
[b] Data from Scuvée-Moreau and Dresse (1979)

For NA uptake, the analysis by the Lineweaver-Burk plot results in a single straight line with a Km value of 5.5×10^{-7} mol/l. This value is similar to the value previously reported by Shaskan and Snyder (1970).

In the case of 5-HT accumulation, the Lineweaver-Burk plot can be resolved into two straight line components. The K_m value for the high affinity component is 10^{-7} mol/l and the low affinity component is 4.7×10^{-6} mol/l. These values are similar to those found by Shaskan and Snyder (1970) in the striatum and in the hypothalamus. The Michaelis-Menten equation was used to calculate the relative contribution of high and low affinity uptakes to the total accumulation of 5-HT into the cortical brain slices in the range of concentrations from 0.02–3.5 mol/l. At the concentration of 0.1 mol/l used in the experiments with antidepressants, the high affinity uptake represents 54% and the low affinity uptake 46% of the total accumulation of 5-HT.

Discussion

Uptake studies were performed on cortical brain slices because this region is rich in both noradrenergic and serotonergic nerve terminals originating from L.C. and D.R. neurones (Fuxe et al. 1968; Lindvall and Björklund 1978; Lidov et al. 1980). Furthermore, according to Shaskan and Snyder (1970), the accumulation of NA and 5-HT into the cortical region indicates a good uptake for both amines.

The results obtained for the inhibition of NA and 5-HT uptake by the tricyclic antidepressants in vitro are in good agreement with uptake studies performed on rat brain synaptosomes by Ross and Renyi (1975a).

Comparison of electrophysiological data with uptake studies performed in vitro shows a good agreement between the order of potency of the five tricyclic antidepressants to inhibit the firing rate of L.C. and D.R. neurones and their respective order of potency to block NA and 5-HT uptake in vitro. The two phenomena seem to be tightly related. The increased availability of neurotransmitter in the synaptic cleft due to uptake blockade probably induces the compensatory decrease of the firing rate of the monoaminergic neurones.

For the noradrenergic neurones, the results obtained show that the inhibition of NA uptake can account at least in part for the inhibition of the firing rate observed after acute administration of the tricyclic antidepressants. In fact, all the

compounds tested at the doses which produce a similar reduction of the firing rate of L.C. neurones cause a significant inhibition of NA uptake into cortical brain slices. It may thus be assumed that the increased availability of NA in the synaptic cleft induces a compensatory decrease in the firing rate of the noradrenergic neurones. According to some experiments performed in our laboratory (Dresse and Scuvée-Moreau 1980; Scuvée-Moreau 1981), different regulatory mechanisms seem to be involved, due to inhibition of uptake at decurrent nerve endings or at recurrent axonal collaterals: 1) a postsynaptic mechanism involving hyperstimulation of α_1 or β adrenoceptors and subsequently the intervention of putative uni- or multineuronal feedback loops; 2) a presynaptic mechanism involving increased stimulation of α_2-presynaptic inhibitory receptors present on L.C. cell bodies (Cedarbaum and Aghajanian 1977). Since the percent inhibition varied between 34 and 50%, it is however possible that uptake inhibition is not the only mechanism involved in the effect of tricyclics on the firing rate of L.C. neurones. For example, one may consider that the α_2-blocking properties of imipramine, clomipramine and amitriptyline (U'Prichard et al. 1978) appear at the doses used and partly reduce the effects on presynaptic inhibitory receptors of increased levels of NA in the synaptic cleft. A greater inhibition of uptake would therefore be required to produce the same effect as desipramine or nortriptyline which possess less α_2-blocking properties. This is only a speculation and other mechanisms could certainly also account for the differences observed.

For the serotonergic neurones, the results obtained ex vivo do not allow us to establish a correlation between electrophysiological data and uptake data. In fact, in most cases, the 50% inhibition of the firing rate of D.R. neurones cannot be related to a significant inhibition of 5-HT uptake. Several possibilities could explain the differences between these results and those obtained with NA: 1) differences in the mode of action of the tricyclic antidepressants on noradrenergic and serotonergic neurones. The inhibition of the firing rate of D.R. neurones would not be related to 5-HT uptake blockade. This explanation does not seem probable in view of the good correlation between electrophysiology and uptake studies in vitro; 2) functional differences between noradrenergic and serotonergic neurones, the latter being perhaps more sensitive to an increased availability of transmitter; 3) regional differences in the inhibition of 5-HT uptake by tricyclic antidepressants: the inhibition of uptake in the cortex could be less than in other brain areas. This explanation is not unlikely as Shaskan and Snyder (1970) demonstrated regional variations in the inhibitory potency of antidepressants on uptake mechanisms; 4) several processes of 5-HT uptake. In fact, kinetic analysis performed by Shaskan and Snyder (1970) in striatum and hypothalamus indicate two components of 5-HT accumulation, representing high and low affinity transport systems. These authors conclude that 5-HT probably accumulates not only in serotonergic neurones but also in catecholaminergic neurones. This nonspecificity could explain the weak inhibition of 5-HT uptake measured with tricyclic antidepressants ex vivo. According to our kinetic experiments performed in the cortical region, NA seems to be accumulated specifically in noradrenergic neurones as there is only one component of its uptake. On the other hand, 5-HT seems to be accumulated not only in serotonergic neurones, but also in other neurones, probably catecholaminergic ones as suggested by Shaskan and Snyder (1970). At the amine concentration used, the low affinity uptake (in catecholaminergic neurones) represents 46% of the total uptake. If, for an unknown reason, antidepressant drugs do not inhibit this uptake ex vivo, the percent inhibition of 5-HT uptake measured in our experimental conditions may not be considered as representing the percent inhibition of uptake in serotonergic neurones. Since 46% of the total 5-HT uptake still persists as an accumulation in catecholaminergic neurones, the observed percent inhibition should be roughly multiplied by two in order to reflect the inhibition of the specific 5-HT uptake in serotonergic neurones. Whatever the explanation, the difference between in vitro and ex vivo results has to be underlined.

Acknowledgements. The authors wish to thank Mrs. Muller N. who typed the manuscript and Mr. Letihon F. for his art work. The Companies Ciba-Geigy, Eli Lilly and Merck Sharp & Dohme which kindly gave us the drugs are also acknowledged.

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Received May 6, 1981/Accepted February 3, 1982