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Stereospecific effect of S (+) and R (—) amphetamine on the release of dopamine and serotonine from « nerve ending synaptosomes » (**)

SUMMARY. — The two enantiomers of amphetamine are tested *in vitro* (nerve ending synaptosomes) on neurotransmitters serotonine and dopamine release. The S(+)-isomer has greater effect than R(−) on both neurotransmitter release. The comparison between our results for release experiments and the results of uptake inhibition obtained by other authors [1, 2, 3] suggests that the uptake inhibition is apparent and amphetamine has only a releasing effect on the two neurotransmitters studied.

RIASSUNTO. — E' stato studiato, usando preparazioni di sinaptosomi, l'effetto dei due antipodi ottici dell'amfetamina sul rilascio dei neurotrasmettitori serotonina e dopamina. L'isomero S(+), nei due casi, è risultato più attivo dello R(−).

Un confronto fra i nostri risultati e quelli riportati in letteratura [1, 2, 3] sull'effetto del farmaco come inibitore della captazione, fa pensare che l'inibizione della captazione da parte dell'amfetamina sia soltanto apparente e che, in realtà, il farmaco agisca solo con effetto rilasciante sui due neurotrasmettitori studiati.

INTRODUCTION

The effect of amphetamine enantiomers on epinephrine is already known [4, 5]: the drug acts by inhibiting the uptake of this neurotransmitter [4], but a releasing effect cannot be observed [5]. So it was proposed that amphetamine has only a blocking effect on the epinephrine carriers but does not enter in nerve endings synaptosomes and does not replace the neurotransmitter.

The aim of this study was to investigate if the same amphetamine mechanism of action could be proposed for dopamine and serotonine (5-hydroxy-tryptamine).

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For this purpose the two isomers are added at different concentrations to a prelabeled synaptosomial crude preparation, according to the method proposed by RAITERI *et al.* [6]. The results show that amphetamine has a releasing effect on the neurotransmitters studied and that the S(+) isomer has a greater effect than the R(−) isomer. The results indicate that the amphetamine mechanism of action on dopamine and serotonine is not the same as that of epinephrine, since in the latter case there is no release. Also in the case of dopamine and serotonine amphetamine action could be clarified by a comparison between RC_{50} and IC_{50} values reported in the literature. The reported IC_{50} values are obtained by contemporary incubation of the drug and of the labeled neurotransmitter, so that release and uptake take place concomitantly during incubation and a discrimination between the two mechanisms (direct release and inhibition of uptake) is impossible from the analysis of only IC_{50} values.

The superfusion technique proposed by Raiteri allows the study of neurotransmitter release in the conditions in which uptake is completely prevented; it is impossible to compare the absolute values of RC_{50} and IC_{50}, since the experimental conditions practised are different; on the contrary a comparison between the isomer ratios obtained by the two methods could be significant: so, obtaining different isomer ratios, it could be proposed that amphetamine acts as releaser and contemporarly as uptake inhibitor, but in the case of similar isomer ratios it is probable that uptake inhibition is only apparent and the drug acts only as releaser.

EXPERIMENTAL

Material and methods

a) Mitochondrial pure fraction [7].

Adult Wistar rats (200-300 g) were sacrified by beheading; the brain was quickly removed and the *corpum striatum* (600-700 mg/rat), diluted in 0.32 M sucrose (10 ml/g; 4° C), was withdrawn and homogenated (twelve crossings; 4° C, 900 r.p.m., potter to pestle distance 0.25 mm). The homogenate was centrifugated at 3,000 r.p.m. (10' at 4° C) and the suspension recentrifugated at 10,000 r.p.m. (20' at 4° C) and washed in the same conditions. The supernatant was discharged and the pellet was suspended in a volume of 0.32 M glucose to obtain, after dissolution of 0.1 ml in 1.9 ml of 2.0% w/v SDS, an O.D. (280 nm) of 0.50; this fraction is usually named mitochondrial pure fraction or "crude".

b) Neurotransmitter uptake by "crude".

0.40 ml of "crude" are suspended in Dubnoff bath for 10' at 37° C in incubation medium adding 0.040 ml of H^3-neurotransmitter to a final concentration of 10^{-7} M. The time of incubation was 10'.

c) Release.

5.0 ml of incubation medium are spilled in a perfusion system od eight elements [4] and, after the addition of 0.4 ml of labeled "crude", laid on Millipore filter (0.6 m Ø). After washing with 15 ml of incubation medium, 6.0 ml of perfusion medium are poured in the perfusion chambers. 0.46 ml fraction/min are collected with a peristaltic pump. After discharging the first three fractions the remaining ones are collected until the 16th min. At the eighth minute, 15 ml of S(+) or R(−) amphetamine in perfusion medium are added to six perfusion chambers leaving two as controls.

At the end of experiment the remaining solution is removed and fractions and filters counted in 15 ml of scintillation cocktail solution *** (β-counter Beckman LS 7500).

Solutions

Incubation medium	Perfusion medium
6 parts H_2O	7 parts H_2O
2 » A solution *	2 » A solution *
1 » B solution **	1 » B solution **
1.25×10^{-5} M nialamide	1.25×10^{-5} M nialamide
1.13 mM ascorbic acid	1.13 mM ascorbic acid
	0.05 M glucose

* A solution: NaCl 0.64 M, KCl 0.025 M, $CaCl_2$ 0.014 M, $MgSO_4$ 0.06 M.

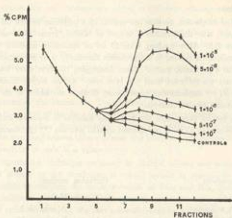
** B solution: Na_2HPO_4 0.05 M in Tris-chloride buffer 0.1 M pH 7.2.

*** Scintillation cocktail solution: 7.0 g of Botyl-BPD in 1.0 l of toluol and 0.6 l of ethylene-glycol Monomethylether.

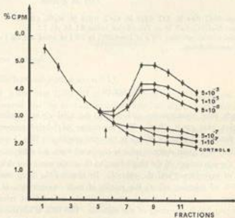
Results

For each drug concentration and control the total c.p.m. result from the c.p.m./fraction plus c.p.m./filter. The percentage of labeled neurotransmitter released in function of minutes of exit is reported graphically (Fig. 1 and 2).

The effect of a given concentration is computed from the difference between the sum of the percentages of the single fraction (from the minute of drug addition to the end of experiment) and the control. To obtain RC_{50} (drug concentration exerting 50% of releasing effect) the results of each concentration of S(+) or R(−) amphetamine are plotted in a semilogarithmic scale (% of release versus log C) assuming 100% the mean of the controls. The RC_{50} is obtained with the least square method obtaining a correlation coefficient varying from 0.96 to 0.99. The absolute error is computed by the ponderal use of standard deviations of every point (mean of almost three experiments) and the trustworthy limit.

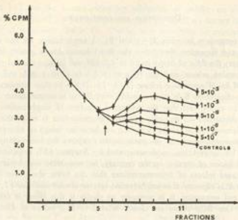


S(+) AMPHETAMINE



R(-) AMPHETAMINE

Fig. 1. — Release of dopamine.



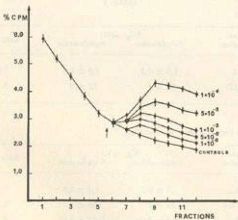


Fig. 2. — Release of serotonine.

Discussion and conclusions

The comparison between S(+) and R(−) amphetamine on the release of serotonine and dopamine shows that the S(+) isomer has a greater effect than R(−) isomer; the RC_{50} of S(+) is 1.0 ± 0.2 μM and of R(−) is 5.0 ± 0.9 μM for H^3-dopamine release and the RC_{50} of S(+) is 22 ± 4 μM and of R(−) 72 ± 9 μM for H^3-serotonine release (Table 1). It can be thus supposed that the absolute configuration S is more suitable than R for neurotransmitter replacing. Besides, due to the fact that the molecular structure of amphetamine is better related to dopamine, the replacing of this neurotransmitter is easier than of serotonine. Moreover, these results show that amphetamine brings an increased availability of neurotransmitter to its postsynaptic receptors by two principal modes of action; for epinephrine, as demonstrated by Raiteri [5], amphetamine acts only by inhibition of uptake; on the contrary, for serotonine and dopamine, there is an increased release of neurotransmitter that can bring about the same final effect. As it is reported that amphetamine acts as uptake inhibitor [1, 2, 3] on dopamine and serotonine, it is necessary to clarify if this effect is real or only apparent. Our RC_{50} values are closely related to IC_{50} values of Holmes and

Table 1

RC_{50} *values*.

Neuro transmitter	RC_{50} (μM) S-amphetamine	R-amphetamine	Ratio R/S
DA	1,0 ± 0,2	5,0 ± 0,9	5,0
5-HT	22 ± 4	72 ± 9	3,3

IC_{50} *values* (reported from the literature).

Neuro transmitter	IC_{50} (μM) S-amphetamine	R-amphetamine	Ratio R/S
$DA_{(1)}$	1,0 ± 0,2	5,4 ± 0,8	5,4
$5\text{-}HT_{(1)}$	28 ± 2	66 ± 12	2,4
$DA_{(2)}$	0,28 ± 0,08	0,85 ± 0,05	4,2
$DA_{(3)}$	0,84 ± 0,4	3,1 ± 0,4	3,6

Rutledge [1] and the isomer ratios for inhibition of uptake are the same as those of other authors [2, 3] (5-fold greater effect for the S(+) isomer in dopamine and 3-fold in serotonine (Table 1). Owing to a possibility in misunderstanding results during the uptake experiments, as the lower radioactive level found on nerve endings may be due to enhanced release of recently accomulated H^3-neurotransmitter, as emphasized by Holmes and Rutledge [1], it can be assumed that probably amphetamine acts on dopamine and serotonine only as a releaser.

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