Biochemical Pharmacology, Vol. 32, No. 15, pp. 2329–2331, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

DOPAMINE UPTAKE INHIBITING VERSUS DOPAMINE RELEASING PROPERTIES OF FENCAMFAMINE: AN *IN VITRO* STUDY

CHRISTOPH A. SEYFRIED*

Department of Experimental Medical Research, Group Biochemical Pharmacology/Neurochemistry, Merck, Darmstadt, West Germany

(*Received* 27 *December* 1982; *accepted* 2 *March* 1983)

Abstract—To elucidate its mechanism of action, the effects of fencamfamine, a norcamphanethylamine derivative, were studied *in vitro* on the release of newly accumulated dopamine in superfusion experiments using slices of rat corpus striatum and substantia nigra in comparison to nomifensine and *d*-amphetamine. Furthermore, the activity of fencamfamine on biogenic amine uptake in synaptosome preparations and on monoamine oxidase activity was evaluated. As compared to *d*-amphetamine, fencamfamine exhibited roughly 10 times less dopamine releasing activity in striatal slices at concentrations at which dopamine uptake into synaptosomes was almost completely blocked although, at high concentrations, what was probably non-specific release due to interactions with neuronal membranes could also be demonstrated. In addition, fencamfamine, unlike *d*-amphetamine did not inhibit monoamine oxidase. It is concluded that, at least in the models employed, the *in vitro* profile of fencamfamine is more similar to that of nomifensine, a reportedly pure uptake inhibitor, than to *d*-amphetamine.

Fencamfamine (2-ethylamino-3-phenyl-norcamphane) is a central stimulant [1] which, according to behavioral studies and theoretical reasoning, should act on central dopaminergic and, possibly, also on noradrenergic neurons. Until now, little was reported about the neurochemical mechanism of action of this drug. Therefore, some basic mechanisms underlying neuronal transmission, i.e. uptake and release, were studied in comparison with standard drugs whose mechanism of action is established.

MATERIALS AND METHODS

Male rats of the Wistar strain, (Wistar WU Kisslegg, Ivanovas GmbH, Kisslegg/Allgäu) of 150–200 g body wt were used. Monoamine oxidase [EC 1.4.3.4. monoamine: oxygen oxidoreductase (deaminating)] activity was determined in whole brain homogenates with [^{14}C]tryptamine [2] ([side chain-2-^{14}C] tryptamine, sp. act. 48.5 mCi/mmole, New England Nuclear). For the release studies, corpus striatum (caudate putamen and globus pallidus) and substantia nigra were carefully dissected [3] and slices made using a McIlwain tissue chopper ($3 \times 3 \times 0.3$ mm for corpus striatum and $0.2 \times 0.2 \times 0.5$ mm for substantia nigra). Slices were incubated in 10^{-7} M [^{3}H]dopamine ([7-^{3}H (N)] dopamine, sp. act. 21.5 Ci/mmole, New England Nuclear), in physiological salt solution for 30 min under an atmosphere of 95% O_2/5% CO_2. The physiological salt solution, which was also used as the superfusion medium, had the following composition (mmole/l.): NaCl 118; KCl 4.8; $CaCl_2$ 1.3; $MgSO_4$ 1.2; $NaHCO_3$ 25; KH_2PO_4 1.2; glucose 11; ascorbic acid 0.57; disodium EDTA 0.03. It was saturated with a mixture of 95% O_2/5% CO_2, the pH was adjusted to 7.4 by addition of NaOH. After incubation, superfusion at 37° was started with drug-free medium at a rate of 0.5 ml/min. In most cases, surface radioactivity from the slices was removed after 15 min and a constant low base line was reached. After this time, superfusion with medium containing the appropriate concentrations of the drug to be tested was initiated for 2 min, thereafter superfusion was continued with drug free medium until base line levels of radioactivity were obtained again. Fractions of 1 ml were collected for radioactivity counting and residual radioactivity of the slices was also determined. Release is expressed in percentage of the total radioactivity originally present in the slices. All other experimental details were as described in the lit. [4, 5]. *In vitro* transmitter uptake into synaptosomes was studied using crude whole brain synaptosomes (P_2-fraction) with [8-^{3}H(N)]dopamine, L-[7-^{3}H(N)]noradrenaline and [1,2-^{3}H(N)]serotonin (New England Nuclear, sp. act. 15, 3.8 and 21.4 Ci/mmole, respectively) at a final concentration of 2×10^{-8} M [6, 7].

Table 1. Inhibition of monoamine oxidase activity in rat whole brain homogenates

	IC_{50} (M)
Fencamfamine	$>>3 \times 10^{-4}$
Nomifensine	$>>3 \times 10^{-4}$
d-Amphetamine	8×10^{-6}

[^{14}C]-Tryptamine was used as substrate at a final concentration of 2×10^{-6} M.

* Proofs and correspondence to: C. A. Seyfried, Department of Experimental Medical Research, Group Biochemical Pharmacology/Neurochemistry, Merck, Frankfurter Str. 250, D-6100 Darmstadt, West Germany.

Table 2. Overflow of ^3H in slices of rat corpus striatum and substantia nigra after pre-loading with [^3H]dopamine at a final concentration of 10^{-7} M

Concentration of drug (M)	Fencamfamine	Nomifensine	*d*-Amphetamine	*n*
Corpus striatum				
10^{-4}	4.05 ± 0.46	2.74 ± 0.32	ND	8
10^{-5}	0.62 ± 0.1	0.57 ± 0.07	12.1 ± 2.65	8
10^{-6}	0.08 ± 0.02	0.057 ± 0.02	1.43 ± 0.99	8
10^{-7}	ND	ND	<0.05	8
Substantia nigra				
10^{-3}	5.84 ± 0.98	3.3 ± 0.3	ND	6
2×10^{-4}	1.43 ± 0.32	0.83 ± 0.35	6.88 ± 0.86	8
10^{-5}	0.1 ± 0.08	1.16 ± 0.43	2.33 ± 0.4	7

Release is expressed as a percentage ± S.E.M. of total radioactivity taken up; *n* = number of rats.

RESULTS

As shown in Table 1, *d*-amphetamine *in vitro* showed a 50% inhibition of monoamine oxidase at a concentration of 8×10^{-6} M, whereas fencamfamine and nomifensine showed virtually no inhibition. At the highest concentration tested (3×10^{-4} M), fencamfamine and nomifensine exhibited inhibitions of only 18 and 25%, respectively, probably due to unspecific interactions with the assay system.

Table 2 summarizes the results of the release experiments. In the striatum, fencamfamine and nomifensine at a concentration of 10^{-6} M released less than 0.1% of the total radioactivity. In contrast, a marked *d*-amphetamine induced ^3H overflow at this concentration was observed which amounted to more than 1%. A similar difference in effects was observed at 10^{-5} M of about 0.5% for fencamfamine and nomifensine vs 12% for *d*-amphetamine. In the substantia nigra, which seemed to be less sensitive than the corpus striatum, *d*-amphetamine again showed pronounced release of about 2% of the accumulated radioactivity. The similarities between fencamfamine and nomifensine were less striking than in the corpus striatum; for fencamfamine 10^{-5} M seemed to be the threshold concentration in the substantia nigra. At concentrations of 10^{-4} M and above, both fencamfamine and nomifensine showed marked release in both areas. However, for comparable effects, the concentrations were 1–2 orders of magnitude higher than with *d*-amphetamine and non-specific release reactions, due to damage of neuronal membranes, probably occur at high concentrations.

As for the *in vitro* uptake inhibition of dopamine and noradrenaline, all drugs were quite active (Table 3), with nomifensine being the most potent followed closely by fencamfamine and *d*-amphetamine being almost equipotent. At 10^{-6} M, dopamine uptake was inhibited by 82, 88 and 76% for fencamfamine, nomifensine and *d*-amphetamine, respectively. As compared to the effects on noradrenaline and dopamine uptake, at least 10 times higher concentrations of all three drugs were necessary to inhibit serotonin uptake by 50%.

DISCUSSION

These results clearly show marked, although quantitative, differences between fencamfamine and *d*-amphetamine. At concentrations of 10^{-6} and 10^{-5} M, which almost completely inhibited synaptosomal dopamine uptake, fencamfamine displayed about 10 times less dopamine releasing activity than *d*-amphetamine. Nomifensine, unlike *d*-amphetamine, has been recently shown to be an uptake inhibitor and not a releaser [4], at least as far as newly accumulated dopamine is concerned. Our findings, using a different model, are in line with this view. *d*-Amphetamine, on the other hand, is generally accepted to be predominantly a releaser rather than an uptake inhibitor [8, 9] although, in the experiments with synaptosomes, *d*-amphetamine and fencamfamine were almost equipotent. Because of the fact that the synaptosome and the slice preparations cannot be compared directly due to different sensitivities, it could be argued that the dopamine uptake inhibition at 10^{-6} M of both fencamfamine and *d*-amphetamine is actually a release phenomenon giving only an apparent uptake inhibition. From the dose–response curve in the slice preparation, however, it is clear that artifactual uptake

Table 3. Uptake inhibition in crude whole brain synaptosomes

	IC_{50} (M)		
	Fencamfamine	Nomifensine	*d*-Amphetamine
NA	6×10^{-8}	2×10^{-8}	8×10^{-8}
DA	10^{-7}	5×10^{-8}	2×10^{-7}
5HT	2×10^{-6}	3×10^{-6}	7×10^{-6}

^3H labelled biogenic amines were used at a final concentration of 2×10^{-8} M.

inhibition, due to release, should play a far greater role with *d*-amphetamine than with fencamfamine. Therefore, it can be concluded that, at least *in vitro*, in concentrations up to 10^{-5} M the predominant actions of fencamfamine seem to be uptake inhibition and not release of dopamine, in contrast to *d*-amphetamine. Another important difference between *d*-amphetamine and fencamfamine is the lack of monoamine oxidase inhibition of the latter.

Acknowledgements—The excellent technical assistance of Helga Spichalski and Peter Tobler is gratefully acknowledged. Nomifensine was a kind gift of Hoechst AG, Frankfurt.

REFERENCES

1. R. Hotovy, H. J. Enenkel, J. Gillissen, A. Hoffmann, U. Jahn, H. G. Kraft, H. Müller-Calgan, S. Sommer and R. Struller, *Arzneimittel-Forsch.* **11**, 20 (1961).
2. H. Parvez and S. Parvez, *J. Neurochem.* **20**, 1011 (1973).
3. T. G. Heffner, J. A. Hartman and L. S. Seiden, *Pharmac. Biochem. Behav.* **13**, 453 (1980).
4. P. Hunt, J.-P. Raynaud, M. Leven and U. Schacht, *Biochem. Pharmac.* **28**, 2011 (1979)
5. R. Jackisch, A. Zumstein, G. Hertting and K. Starke, *Naunyn-Schmiedebergs Arch. Path. exp. Pharmak.* **314**, 129 (1980).
6. M. H. Kannengiesser, P. Hunt and J.-P. Raynaud, *Biochem. Pharmac.* **22**, 73 (1973).
7. D. T. Wong, J.-S. Horng and R. W. Fuller, *Biochem. Pharmac.* **22**, 311 (1973).
8. R. E. Heikkila, H. Orlansky, C. Mytilineou and G. Cohen, *J. Pharmac. exp. Ther.* **194**, 47 (1975).
9. M. Raiteri, A. Bertollini, F. Angelini and G. Levi, *Eur. J. Pharmac.* **34**, 189 (1975).