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J. Pharm. Pharmacol. 1990, 42: 437–438
Communicated December 11, 1989

The effect of nefopam and its enantiomers on the uptake of 5-hydroxytryptamine, noradrenaline and dopamine in crude rat brain synaptosomal preparations

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Abstract—The effect of (±), (+) and (−)-nefopam on the uptake of 5-hydroxytryptamine (5-HT), noradrenaline and dopamine in synaptosomal preparations from rat forebrain, hippocampus and striatum has been investigated. All three forms of nefopam inhibited the amine uptake in the investigated structures, the order of potency being (+) > (±) > (−). (+)-Nefopam was 7–30 times more potent than (−)-nefopam. The same order of potency has also been found for the antinociceptive effect of these three forms, however, the differences were smaller. Inhibition of 5-HT and noradrenaline uptake may not be the sole mechanism underlying the analgesic effect of nefopam.

Racemic nefopam has analgesic effects in humans (for review, see Heel et al 1980), and has also been shown to have antinociceptive effects in a broad range of animal tests (Conway & Mitchell 1977; Piercey & Schroeder 1981; Fasmer et al 1987). Hunskaar et al (1987) have demonstrated that the antinociceptive effect may be mediated, at least partly, via raphe-spinal 5-hydroxytryptaminergic systems.

It has previously been shown that the different enantiomers of nefopam clearly differ in their antinociceptive effects, the order of potency being (+)-nefopam > (±)-nefopam > (−)-nefopam (Fasmer et al 1987).

Nefopam inhibits the synaptosomal uptake of 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA) (Tresnak-Rustad & Wood 1981). This uptake inhibition may be related to the analgesic effect. If this hypothesis is correct, one would expect that the enantiomers would show a similar order of potency in uptake inhibition as in antinociceptive activity.

The aim of the present study was to study the effect of nefopam and its enantiomers on the synaptosomal uptake of [^{14}C]5-HT, [^{3}H]NA and [^{3}H]DA in-vitro. Crude synaptosomal preparations were prepared from the rat forebrain, as well as from the striatum, where there are 5-HT and dopaminergic, but few noradrenergic synaptosomes, and from the hippocampus, where there are 5-HT and noradrenergic, but few dopaminergic synaptosomes.

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Methods

Male Sprague-Dawley rats (Mol: SPRD, Møllegaard, Denmark) were decapitated during the third or fourth hour of the light period. The brains were rapidly removed and cooled on ice. Forebrain (rostral to cerebellum), hippocampus or striatum were dissected out and crude synaptosomal preparations prepared. The tissue was homogenized in 10 volumes of 0·25 M sucrose. The homogenate was centrifuged (100 *g*, 0°C, 10 min), and 0·075 mL of the supernatant (containing synaptosomes) was added to a modified Krebs-Ringer bicarbonate buffer (pH 7·3) to make a final volume of 0·7 mL. After preincubation at 37°C for 3 min, [^{14}C]5-HT, [^{3}H]NA, or [^{3}H]DA was added to give a final concentration of 10 nM of [^{3}H]NA or [^{3}H]DA, and 100 nM of [^{14}C]5-HT (Fasmer et al 1985). The incubation was continued for 10 min and terminated by rapid cooling on ice followed by filtration in a Titertec cell harvester. The filters (Whatman GF/B) containing synaptosomes, were washed with ice-cold 0·9% NaCl and transferred to counting vials; 4 mL of scintillation fluid (Insta-Gel, Packard) was added. The samples were analysed in an LKB Wallac 1219 Rack Beta Spectral liquid scintillation counter. Non-specific amine uptake was determined in control samples in the presence of cocaine (1 mM), and the values subtracted from the total uptake.

Results and discussion

Results were obtained for the uptake of [^{14}C]5-HT in synaptosomal preparations from rat forebrain, hippocampus and striatum, for the uptake of [^{3}H]NA in preparations from forebrain and hippocampus, and for [^{3}H]DA in preparations from striatum. (+)-Nefopam, (±)-nefopam as well as (−)-nefopam inhibited uptake of all three amines in all structures investigated. The uptake in synaptosomal preparations from forebrain for [^{14}C]5-HT and [^{3}H]NA are shown in Fig. 1 and Fig. 2, respectively, and the IC50 values for all structures are shown in Table 1.

For all structures, and for all three amines, the order of potency for the uptake inhibition was (+)-nefopam > (±)-

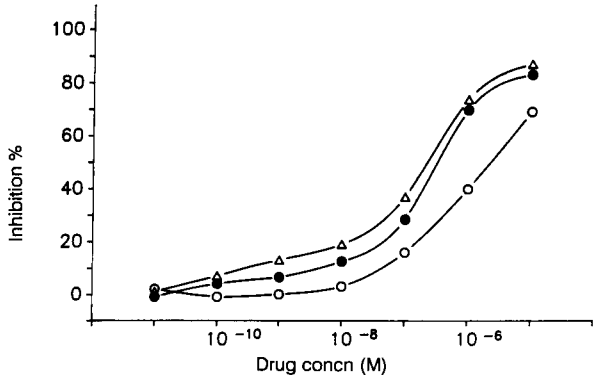

FIG. 1. The inhibition of the uptake of [^{14}C]5-HT into crude synaptosomal preparations from forebrain by (±) ●——●, (+) △——△ and (−)-nefopam ○——○. Each data point is the mean of three determinations.

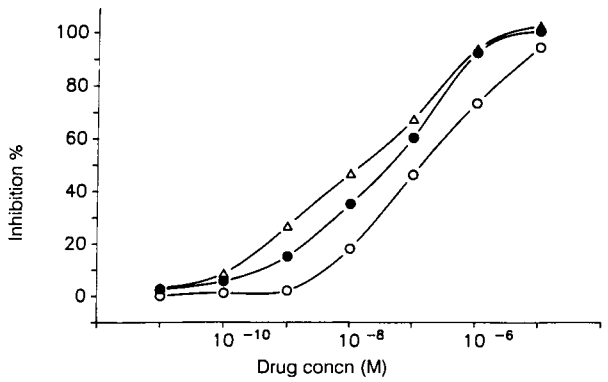

FIG. 2. The inhibition of the uptake of [^{3}H]NA into crude synaptosomal preparations from forebrain by (±) ●——●, (+) △——△, and (−)-nefopam ○——○. Each data point is the mean of three determinations.

nefopam > (−)-nefopam. (+)-Nefopam was a potent uptake inhibitor for all three amines. For the [^{3}H]NA uptake the IC50 values were particularly low, lower in the hippocampus (6 nM) than in the whole forebrain (15 nM). (+)-Nefopam was 7–30 times more potent than (−)-nefopam (all structures and all three amines), indicating that most of the uptake inhibition exerted by (±)-nefopam was due to the (+)-nefopam.

The ranked order of potency for the antinociceptive effect of nefopam and its enantiomers in mice (Fasmer et al 1987) is the same as that reported here for the uptake inhibition, although the differences are not so marked. This, coupled with the observation that some other potent inhibitors of 5-HT and NA uptake are less effective than nefopam as analgesics (Magni et al 1987; Eide & Hole 1988; Rosland et al 1988) would suggest that the uptake inhibition of amines is not the sole mechanism of the antinociceptive effect. However, differences in pharmacokinetics resulting in different concentrations at the tissue in in-vivo experiments cannot be ruled out.

Table 1. The concentrations in nM of (+)-nefopam, (−)-nefopam and (±)-nefopam inducing 50% inhibition (IC50) of the uptake of [^{14}C]5-HT, ^{3}[H]NA, [^{3}H]DA, in crude synaptosomal preparations from forebrain, hippocampus, or striatum. Means of six determinations ± s.e.m. (+) = (+)-nefopam; (−) = (−)-nefopam; (±) = (±)-nefopam. Statistically significant differences compared to (±)-nefopam are indicated by $*P < 0.01$ and $**P < 0.001$, two tailed *t*-test.

		[^{14}C]5-HT	[^{3}H]NA	[^{3}H]DA
Forebrain	(+)	199 ± 6**	15 ± 1*	
	(±)	354 ± 17	36 ± 5	
	(−)	1930 ± 185**	141 ± 12**	
Hippocampus	(+)	309 ± 16**	6 ± 1**	
	(±)	599 ± 17	13 ± 1	
	(−)	2988 ± 76**	197 ± 7**	
Striatum	(+)	247 ± 17**		800 ± 14**
	(±)	496 ± 19		1505 ± 49
	(−)	1994 ± 31**		6035 ± 93**

Nefopam and its enantiomers were generously supplied by 3M-Health Care Limited. J. H. Rosland is a Fellow of the Norwegian Cancer Society—Den Norske Kreftforening.

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