# Inhibition of rat synaptosomal catecholamine uptake by niflumic acid and indomethacin

P. CLARENBACH, G. RAFFEL, D. K. MEYER, G. HERTTING*, *Department of Pharmacology, University of Freiburg i.Br., D 7800 Freiburg, FRG*

Recently it has been shown that incubation of rat brain synaptosomes at more than 10° triggers off the synthesis and release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), the rate of which is temperature dependent (Clarenbach, Raffel & others, 1974). In the peripheral sympathetic nerve, $PGE_2$ has an inhibitory function on the release of noradrenaline (Hedqvist, 1970) and in the brain a similar, although less pronounced effect was observed by Bergstrom, Farnebo & Fuxe (1973) and by Starke & Montel (1973). The catecholamine-uptake into a synaptosomal preparation was not affected by exogenous $PGE_1$, $PGE_2$ and $PGF_{2\alpha}$ (Ciofalo, 1973). Since PG's are rapidly metabolized and may not reach their sites of action when given exogenously, we have investigated the possible role of endogenous prostaglandins in the uptake of catecholamines.

Adult male rats (Wistar), 250–350 g, were killed by cervical dislocation and the brains rapidly removed and the cerebella discarded. Synaptosomal fractions of whole brain tissue were prepared by the method of Gray & Whittaker (1962). A crude nuclei fraction was obtained by centrifugation of brain homogenate in 0·32 M sucrose at 1000 *g* for 10 min. The supernatant was further sedimented at 17 000 *g* for 1 h to obtain pellet $P_2$. Synaptosomes were further purified by discontinuous sucrose gradient centrifugation. The 1·2–0·8M interphase was aspirated and sucrose concentration was adjusted to 0·32 M. It was then resedimented at 35 000 *g* for 15 min to obtain the synaptosomal fraction, which was resuspended in isotonic medium to give a protein concentration of 5–8 mg $ml^{-1}$ (Lowry, Rosebrough & others, 1951). The incubation medium contained (mM): $Na^+$ 145, $K^+$ 5, $Cl^-$ 105, phosphate 28, glucose 10, sucrose 40, nialamid 12·5 $\mu$M and ascorbic acid 140 $\mu$M; the pH was adjusted for 7.

Incubations were in a shaker at 37°. Incubation tubes contained 0·2 ml of synaptosomal suspension, 0·6 ml of medium, 0·1 ml of solvent or drug, and 0·1 ml of labelled substrate of varying specific activity. Incubations were stopped by the addition of 3 ml of chilled medium immediately followed by ultrafiltration. Filters were dissolved in cellosolve (ethylene glycol-monoethylether) and submitted to liquid scintillation spectrometry. The results are expressed as velocity = pmol/incubation period × mg protein. Kinetic analysis of synaptosomal amine uptake and its inhibition was according to Lineweaver & Burk (1934) and Dixon (1953).

* Correspondence.

For the investigation of the spontaneous release of [$^3$H]noradrenaline ($^3$H-NA) and [$^3$H]dopamine ($^3$H-DA) from synaptosomes, pellet $P_2$ was loaded for 15 min with 300 nCi $^3$H-NA or $^3$H-DA per mg protein (specific activities: $^3$H-NA = 5·6 Ci $mmol^{-1}$, 3H-DA = 6·0 Ci $mmol^{-1}$). After centrifugation at 14 000 *g* for 15 min, resuspension in medium and incubation with or without drug at 37°, the samples were rapidly ultrafiltrated and the filters treated according to the uptake procedure.

The release of $PGF_2$ in aliquots of the same preparations, which were used for the investigation of amine uptake, was studied as reported by Clarenbach & others (1974). $PGF_2$ was determined by a specific radioimmunoassay (Peskar & Hertting, 1973; Jobke, Peskar & Peskar, 1973; Liebig, Bernauer & Peskar, 1974).

Fig. 1 shows the time-dependence of the accumulation of noradrenaline ($5·5 \times 10^{-8}$M) and dopamine ($6·6 \times 10^{-8}$M) at 37°. The period of initial velocity was 1·5 min for noradrenaline and 2·0 min for dopamine. These incubation periods were used for most experiments.

Experiments were performed within the range of high affinity uptake (Km for noradrenaline: $5 \times 10^{-7}$M; Km for dopamine: $2·4 \times 10^{-7}$M). Niflumic acid non-competitively inhibited the accumulation of both amines; Ki for noradrenaline was $7·6 \times 10^{-5}$M, Ki for dopamine $1·08 \times 10^{-4}$M.

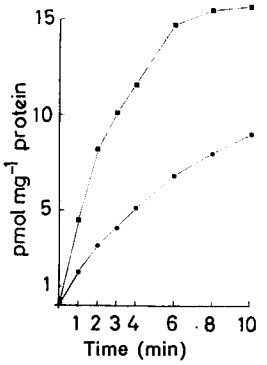


FIG. 1. Time course of uptake of ■ – ■ – noradrenaline ($5·5 \times 10^{-8}$M) and ● – ● dopamine ($6·6 \times 10^{-8}$M) into rat brain synaptosomes at 37° after 4 min of preincubation.

The effect of indomethacin was more complex, as it caused a non-competitive inhibition of the accumulation of noradrenaline ($Ki = 8 \times 10^{-5}$M), but a competitive inhibition of the dopamine-uptake ($Ki = 4{\cdot}4 \times 10^{-5}$M).

Low affinity uptake of both amines (Km for noradrenaline $= 2{\cdot}5 \times 10^{-6}$M, Km for dopamine $= 2 \times 10^{-6}$M) was not affected by the drugs studied.

The effect of both drugs on amine-uptake could not be explained by a concomitant action on amine-release, as $5 \times 10^{-5}$M niflumic acid and indomethacin, respectively, had no effect on the spontaneous release of $^3$H-NA or $^3$H-DA.

Whether the effect of both drugs was mediated by their inhibitory action on prostaglandin synthesis or independently of this by an action directly on the amine pump required investigation. A comparison of the ID50 values for the inhibition of PG synthesis (niflumic acid $1{\cdot}5 \times 10^{-7}$M; indomethacin $3 \times 10^{-7}$M) and their Ki values for inhibition of amine-uptake favours the latter assumption, which finds further support by the following experiments:

(1) Synaptosomes were preincubated for 7 min at 37° when PG synthesis had almost reached its maximum: the level of $PGF_{2\alpha}$ was taken 100%. Further incubation with $^3$H-DA for 4 min resulted in an accumulation of dopamine, which was also taken 100%.

(2) In a second experiment PG synthesis was blocked by $5 \times 10^{-5}$M indomethacin and dopamine uptake determined. It was reduced to 60%.

(3) In a third experiment, indomethacin was added when the termination of PG synthesis already had been reached. Under this condition dopamine uptake was the same as in the second experiment, i.e. 60% of the control uptake. The same proved to be true for niflumic acid.

It can be concluded, therefore, that the action of indomethacin and niflumic acid on PG synthesis and amine uptake are two separate effects not causally related.

The PG synthesis inhibitors niflumic acid and indomethacin showed an inhibition of catecholamine accumulation into rat brain synaptosomes without affecting the spontaneous release of both amines. There is, however, strong evidence that this action of niflumic acid and indomethacin is independent of their inhibitory effect on PG synthesis:

(1) The ID50 values for PG synthesis inhibition by niflumic acid and indomethacin were $1{\cdot}5$ and $3 \times 10^{-7}$M, respectively, whereas the Ki values for uptake of noradrenaline and dopamine ranged between 4·4 and $10{\cdot}8 \times 10^{-5}$M. The doses necessary for the inhibition of catecholamine-uptake, therefore, were much higher than for inhibition of PG synthesis.

(2) At lower doses of niflumic acid and indomethacin, which completely inhibited PG synthesis, the catecholamine-uptake was practically unchanged.

(3) Niflumic acid and indomethacin inhibited catecholamine uptake whether PG synthesis had taken place or not.

Thus, it was concluded that inhibition of PG synthesis in rat brain synaptosomes and inhibition of catecholamine uptake were two distinct, independent effects of both niflumic acid and indomethacin.

Niflumic acid displayed an inhibition of the non-competitive type for the uptake of both amines. This indicates that the drug does not inhibit the access of the amines to the sites of transport, but acts on another part of the amine pump. The action of indomethacin, however, is more complex. It causes a non-competitive inhibition of noradrenaline uptake, but inhibits dopamine uptake competitively. Thus it antagonizes noradrenaline uptake possibly in a similar manner to niflumic acid, whereas it diminishes the access of dopamine to the membrane pump. Speculations on the mechanism of this aspect of the action of indomethacin are hampered by the fact that the whole brain synaptosomal preparation we used contains noradrenaline as well as dopamine nerve endings from the different brain regions.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 70).

September 22, 1975

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