J. Pharm. Pharmacol. 1979, 31: 818–821 Received March 19, 1979

# Release of [$^3$H]noradrenaline induced by 5-hydroxytryptamine from cat pial arteries

J. MARIN*, M. SALAICES AND C. F. SÁNCHEZ

*Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma Madrid-34, Spain*

Pial arteries of cats were used to analyse the effects of 5-hydroxytryptamine (5-HT) on the release of [$^3$H]noradrenaline. To achieve this the vessels were preincubated with [$^3$H]noradrenaline and the effect of different concentrations of 5-HT ($10^{-6}$, $10^{-5}$, $10^{-4}$ M) on the release of tritium was studied. 5-HT elicited release of radioactivity in a dose-dependent manner. Removal of both superior cervical sympathetic ganglia 15 days before the experiment or pretreatment of the animals with reserpine (3 mg kg$^{-1}$, total dose) produced a significant decrease in the outflow of tritium induced by 5-HT. In these arteries, the amount of radioactivity retained at the end of the experiment was much diminished. Cocaine ($10^{-6}$ M) caused a significant decrease in the tritium efflux induced by 5-HT ($10^{-5}$ M). These results show that 5-HT has an indirect adrenergic effect in the pial arteries of the cat only at high doses of 5-HT, and confirm that sympathetic innervation of these vessels mainly comes from the superior cervical ganglia.

Pial vessels have adrenergic innervation which has its origin in the superior cervical sympathetic ganglia (Nielsen & Owman 1967; Iwayama 1970; Edvinsson et al 1975; Alborch et al 1977). On the other hand, the presence of specific receptors for 5-hydroxytryptamine (5-HT) which mediate the vasoconstrictor effects of this drug, has been demonstrated in the cerebral blood vessels (Allen et al 1974b; Urquilla et al 1975; Edvinsson et al 1978; Marin et al 1979). Nevertheless, the mechanism of action of 5-HT is not yet clear because, in addition to its direct activation of these tryptaminergic receptors, there is evidence that 5-HT might have an indirect adrenergic component in its overall actions on different tissues. Thus, 5-HT releases noradrenaline from dog and rabbit hearts (Fillion et al 1971; Fozard & Mwaluko 1976), and vascular smooth muscle (McGrath 1977). Indirect evidence of this effect is also seen in experiments with unanesthetized goats showing that the decrease in cerebral blood flow produced by 5-HT is partially abolished by phentolamine or reserpinization (Lluch et al 1976). In addition, the vasospasm induced in the dog by intracisternal administration of 5-HT may be reversed by phenoxybenzamine (Allen et al 1974a). Nevertheless, the contractile response induced by 5-HT on the posterior communicating artery of the cat seems to be mainly due to the direct activation of tryptaminergic receptors (Marin et al 1979).

The aim of the present study is to analyse whether 5-HT has the ability to release noradrenaline from pial arteries of the cat and its modification by different procedures which alter the sympathetic activity of these vessels.

* Correspondence: Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma, c/ Arzobispo Morcillo s/n, Madrid-34, Spain.

## MATERIALS AND METHODS

Cats of either sex, 1·5–4 kg, were anaesthetized with sodium pentobarbitone 35 mg kg$^{-1}$ (i.p.) and bled to death. The brain was removed and the Willis circle arteries with their ramifications were dissected and placed in a Petri dish, which contained Krebs-Henseleit (K-H) solution at 4 °C, and the blood of the arteries removed. Twenty normal, 3 denervated and 3 reserpinized cats were used to study the release of radioactivity from [$^3$H]noradrenaline ($^3$H-NA) preloaded tissues.

The blood vessels were placed in a cylindrical nylon net and preloaded with $^3$H-NA as follows: about 10–20 mg, which corresponded to the pial arteries of a cat, were pooled in one beaker containing 4 ml of oxygenated K-H solution. After 15 min equilibration at 37 °C, tissues were exposed to 1-$^3$H-NA ($2{\cdot}10^{-7}$ M, 2 $\mu$Ci ml$^{-1}$, specific activity 9·1 Ci mmol$^{-1}$) for 30 min and continually washed with fresh K-H solution at 10 min intervals during 100 min. To estimate spontaneous tritium release, the arteries were successively immersed in 5 vials containing 2 ml of fresh K-H solution for 3 min periods. 5-HT evoked release of radioactivity was studied by sequentially transferring the tissue to another 4 vials containing 3 ml of K-H with 5-HT in appropriate concentrations; finally the arteries were again exposed to fresh K-H in another 5 vials, to recover the basal level of tritium efflux. Total radioactivity present in the medium was analysed by

adding 0·5 ml of each sample to 10 ml of Bray's solution (Bray 1960) and it was measured in a Nuclear Chicago liquid scintillation spectrometer model ISOCAP 300, using the external standard method to correct for quenching.

At the end of the experiment the arteries were blotted, weighed, homogenized in 0·5 ml of 0·4 N perchloric acid, centrifuged for 15 min at 2000 *g* and an aliquot (0·1 ml) of the supernatant was measured as previously described for the media.

In other experiments, the spontaneous tritium efflux in these vessels was also estimated in a 210 min period. During this time the arteries were washed out with 4 ml of fresh K-H solution each 10 min and the radioactivity present in the media measured.

The composition of the Krebs-Henseleit solution was (mM); NaCl, 115; KCl, 4·6; $CaCl_2$, 2·5; $KH_2PO_4$, 1·2; $MgSO_4 \cdot 7H_2O$, 1·2; $NaHCO_3$, 25; Glucose, 11·1; ethylenediamine tetracetic acid (EDTA, $3 \times 10^{-5}$M), was added to prevent oxidation of unstable substances. This solution was equilibrated with 5% $CO_2$ in oxygen and the final pH was between 7·4 and 7·5.

Reserpine (3 mg $kg^{-1}$, i.p., total dose) was administered 2 mg $kg^{-1}$ and 1 mg $kg^{-1}$, 48 and 24 h respectively before the experiment.

Cats, previously injected with 0·5 mg $kg^{-1}$ of atropine, were anaesthetized with 35 mg $kg^{-1}$ of pentobarbitone and under aseptic conditions both superior cervical sympathetic ganglia were removed. All the experiments performed with denervated cerebral arteries were carried out 14 or 16 days after the operation at a time when the adrenergic nerve endings had already degenerated (Edvinsson et al 1975; Lee et al 1976). When used, cocaine was present 10 min before and during the period of exposure to 5-HT.

The drugs used were: Atropine sulphate (Sigma); reserpine (Ciba); 7 (−)-[$^3$H]noradrenaline (Radiochemical Centre, Amershan, England); Cocaine hydrochloride (Abelló). Statistical analysis was by means of Student's *t*-test (Snedecor 1956); a probability value of less than 5% was considered significant.

## RESULTS

The spontaneous tritium release shows a two-stage pattern (Fig. 1). After an initial rapid decay of radioactivity during the first 100 min, a second period followed in which approximately a steady state tritium release was reached.

Fig. 2 shows the effect of different concentrations of 5-HT ($10^{-6}$, $10^{-5}$ and $10^{-4}$ M) on the tritium efflux

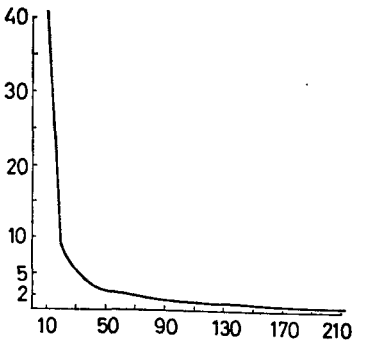


FIG. 1. Spontaneous tritium efflux from cat pial arteries. Abscissa: time (min) after an initial incubation period with $^3$H-NA ($2 \times 10^{-7}$ M) during 30 min and a subsequent washing period of 210 min. Ordinate: release of tritium (count $min^{-1}$ $mg^{-1}$ of tissue). Vials containing 4 ml of medium were collected every 10 min. The graph was plotted from one typical experiment out of five.

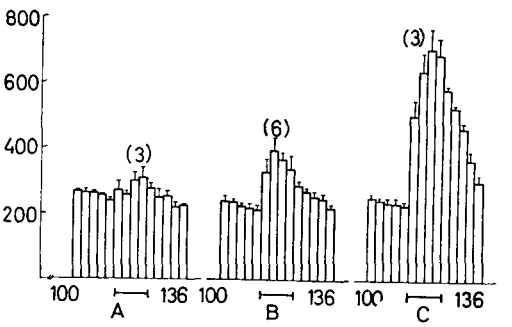


FIG. 2. Comparison between tritium release induced by different concentrations of 5-HT ($10^{-6}$, $10^{-5}$, $10^{-4}$ M, A, B, C respectively) in pial arteries of control cats. Abscissa: time (min) after an initial incubation period with $^3$H-NA ($2 \times 10^{-7}$ M) during 30 min and a subsequent wash out period of 100 min. Each column represents the efflux of tritium during a period of 3 min. Ordinate: release of tritium (count $min^{-1}$ $mg^{-1}$ tissue). Vials containing 2 ml of medium were collected every 3 min. Different concentrations of 5-HT were maintained in the bath for 12 min (horizontal bar). Number of experiments are shown in parentheses. Vertical bars represent s.e. of the means.

from pial arteries of the cat. This efflux was clearly increased by $10^{-4}$ M 5-HT and to a lesser extent by $10^{-5}$ M 5-HT. The basal release of radioactivity was recovered 6 min after the wash out of this amine.

After denervation, the spontaneous tritium outflow and the efflux of radioactivity induced by 5-HT $10^{-5}$ M was considerably diminished ($P < 0.02$) (Fig. 3A). However, in this figure it can be seen there was a slight but significant ($P < 0.05$) increase in the release of radioactivity after the addition of 5-HT ($10^{-5}$ M). The release returned to basal levels after

removal of the drug. In arteries from reserpinized animals, similar results were obtained, i.e., the basal outflow of tritium and the outflow induced by 5-HT was greatly reduced ($P < 0.02$) (Fig. 3B). When cocaine ($10^{-6}$ M) was added to block the neuronal uptake of amines, the tritium release induced by 5-HT was significantly diminished compared with the control ($P < 0.05$) (Fig. 4). The amount of radioactivity retained by these tissues was measured at the end of the experiment. There was a significant difference between the tritium present in pial arteries of normal cats and that present in denervated ($P < 0.001$) and reserpinized ($P < 0.001$) cats (Table 1).

Table 1. Radioactivity retained in pial arteries of the cat after incubation with $^3$H-NA ($2 \times 10^{-7}$ M), wash out with Krebs-Henseleit solution during 100 min and treatment with 5-HT ($10^{-5}$ M) during 12 min. The results are means ±s.e.

| Treatment | Counts min$^{-1}$ mg$^{-1}$ | Retention % | N |
|---|---|---|---|
| Control | 1·945 ± 161 | 100 | 5 |
| Reserpine | 511 ± 7* | 26·3 | 3 |
| Denervated | 204 ± 1* | 10·5 | 3 |

N = Number of experiments.
* $P < 0.001$.

## DISCUSSION

It is generally accepted that adrenergically innervated tissues incubated with exogenous $^3$H-NA take up the amine into adrenergic nerve endings where it is mixed with the endogenous noradrenaline pool. From this pool, the labelled amine can be released by nerve stimulation or by drugs which are capable of displacing it from the specific storage sites (Iversen 1967; Fillion et al 1971; Nedergaard & Schrold 1973).

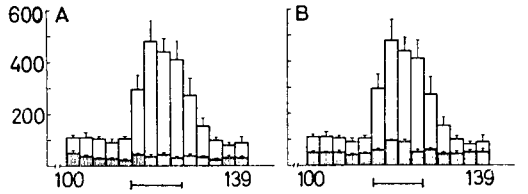


FIG. 3. Tritium overflow induced by 5-HT ($10^{-5}$ M) in cerebral arteries from (A) ganglionectomized (3 experiments) and (B) reserpinized cats (3 experiments) (filled bars) compared with control animals (5 experiments in both A and B) (open bars). The other symbols are similar to those in Fig. 2. Ordinate: tritium release (counts min$^{-1}$ mg$^{-1}$). Abscissa: time (min).

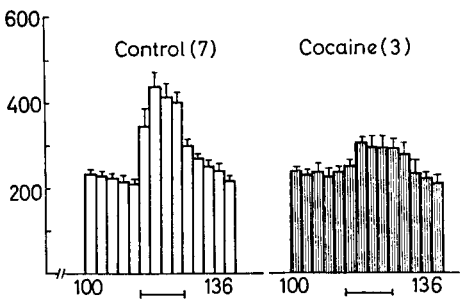


FIG. 4. Effect of $10^{-6}$ M cocaine on the tritium efflux induced by 5-HT ($10^{-5}$ M) from cat pial arteries. The other symbols are similar to those in Fig. 2. Ordinate: tritium release (counts min$^{-1}$ mg$^{-1}$).

On the other hand, the behaviour of 5-HT as an indirectly acting sympathomimetic amine has been demonstrated in several tissues. Thus, Fillion et al (1971) reported that in dog heart previously labelled with $^3$H-NA, the administration of 5-HT elicited a dose-dependent release of radioactivity. McGrath (1977) observed that in extracranial vessels of the dog preincubated with $^3$H-NA, tritiated compounds were released by high doses of 5-HT. In addition, Fozard & Mwaluko (1976) showed in rabbit isolated heart that the cardiac effects of 5-HT and tyramine were inhibited by propranolol, a $\beta$-adrenoceptor blocking agent. Furthermore, they observed that the cardiac responses to tyramine and 5-HT were greatly reduced in hearts pretreated with 6-hydroxydopamine (an agent which specifically destroys the sympathetic nerve endings). When they perfused the normal heart with $^3$H-NA, both 5-HT and tyramine elicited increases in the release of tritiated compounds.

Our results show that the spontaneous tritium efflux from pial arteries of cat preloaded with $^3$H-NA follows a kinetic pattern, upon washing with K-H solution with a fast initial component and a slow component until a steady state is reached. These results are in agreement with those obtained by other investigators in extracranial arteries (George & Leach 1975; Eckert et al 1976). In addition, 5-HT induced a release of radioactivity which was dose-dependent (Fig. 2). The tritium efflux evoked by 5-HT, and the spontaneous outflow in arteries from reserpinized and ganglionectomized cats, was much smaller than in control vessels. These results are similar to those obtained by Edvinsson et al (1977) in the pial arteries of the cat preloaded with $^3$H-NA, in which the tritium outflow induced by electrical stimulation was much diminished by sympathetic denervation. Moreover, the tritium present in these arteries was significantly reduced in denervated and

reserpinized segments compared with control vessels (Table 1). The fact that ganglionectomy diminished the amount of radioactivity retained by these arteries confirms the results already obtained by others who have demonstrated that sympathetic innervation of pial arteries arises from cervical sympathetic ganglia (Nielsen & Owman 1967; Iwayama 1970; Edvinsson et al 1975). On the other hand, cocaine reduced the tritium release induced by 5-HT. This is consistent with the findings of other workers. McGrath (1977) showed that cocaine will inhibit the release of $^3$H-NA by 5-HT in dog isolated saphenous vein and Humphrey (1978) showed that cocaine will antagonize the indirectly mediated contractile effects of 5-HT in this preparation.

The ability of 5-HT to release noradrenaline from pial vessels of the cat shown in the present work is apparently in contradiction with results obtained on communicating cerebral artery of the cat (Marín et al 1979). The results obtained by these investigators indicated that in the vasoconstrictor effects induced by 5-HT there was a small participation, if any, of an adrenergic component. An explanation for this discrepancy can be found in the fact that noradrenaline induces a poor vasoconstrictor response in brain vessels compared with 5-HT (Nielsen & Owman 1971; Toda & Fujita 1973; Allen et al 1974b; Urquilla et al 1975; Marín & Salaices 1978) and that the amount of noradrenaline released by 5-HT is small. Therefore, the contribution of the indirect component of 5-HT to its overall vasoconstrictor effect in the cat communicating cerebral artery is of minor importance compared with the direct effect of 5-HT on tryptaminergic receptors present in this vascular bed. Moreover, it should also be considered that high doses of 5-HT were needed to show clearly its indirect effect, since at $10^{-6}$ M the release of noradrenaline was small, while the contractile response was large (Marín et al 1979).

In conclusion, 5-HT elicits a dose-dependent release of noradrenaline from pial arteries of the cat preloaded with $^3$H-NA. Nevertheless, this effect is only relevant at high doses of 5-HT (greater than $10^{-6}$ M). The reserpinization and denervation greatly reduced the tritium outflow and also the amount of radioactivity retained by these arteries.

*Acknowledgements*

This work was supported by Comisión Asesora de Investigación Científica y Técnica, Presidencia del Gobierno and Instituto Nacional de Previsión, Seguridad Social, Madrid, Spain. We thank Dr. A. G. García for critically reviewing this manuscript.

REFERENCES

Alborch, E., Gomez, B., Dieguez, G., Marin, J., Lluch, S. (1977) Circulation Res. 41: 278–282

Allen, G. S., Gold, L. H. A., Chou S. N. French L. A. (1974a) J. Neurosurg. 40: 451–458

Allen, G. S., Henderson, L. M., Chou, S. N., French, L. A. (1974b) Ibid. 40: 442–450

Bray, G. A. (1960) Anal. Biochem. 1: 279–285

Eckert, E., Henseling, M., Gescher, A., Trendelenburg, U. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 292: 219–229

Edvinsson, L., Aubineau, P., Owman, C., Sercombe, R., Seylaz, J. (1975) Stroke 6: 525–530

Edvinsson, L., Falck, B., Owman, C. (1977) J. Pharmacol. Exp. Ther. 200: 117–126

Edvinsson, L., Hardebo, J. E., Owman, C. (1978) Circulation Res. 42: 143–151

Fillion, G. M. B., Lluch, S., Uvnäs, B. (1971) Acta Physiol. Scand. 83: 115–123

Fozard, J. F., Mwaluko, G. M. P. (1976) Br. J. Pharmacol. 57: 115–123

George, A. J., Leach, G. D. (1975) Biochem. Pharmacol. 24: 737–741

Humphrey, P. P. A. (1978) Br. J. Pharmacol. 63: 671–675

Iversen, L. L. (1967) Uptake and storage of noradrenaline in sympathetic nerves. pp. 147–198: Cambridge University Press

Iwayama, T. (1970) Z. Zellforsch. Mikroskop. Anat. 109: 465–480

Lee, T. J. F., Su, C., Bevan, J. A. (1976) Circ. Res., 39: 120–126

Lluch, S., Dieguez, G., Alborch, E., Ruiz, M. C., Gomez, B. (1976) in Cervós-Navarro, J., Betz, E., Matakas, F. & Wüllenweber, R. (eds) The cerebral vessel wall. Raven Press: New York, pp 135–138

Marin, J., Salaices, M. (1978) Rev. Esp. Fisiol. 34: 449–452

Marin, J., Salaices, M., Marco, E. J., Gomez, B., Lluch, S. (1979) J. Pharm. Pharmacol. 31: 456–459

McGrath, M. A. (1977) Circulation Res. 41: 428–435

Nedergaard, O. A., Schrold, J. (1973) Acta Physiol. Scand. 89: 296–305

Nielsen, K. C., Owman, C. (1967) Brain Res. 6: 773–776

Nielsen, K. C., Owman, C. (1971) Ibid. 27: 33–42

Snedecor, G. W. (1956) Statistic1 Methods, 5th. Edn. Ames. Iowa. U.S.A.: University Press

Toda, N., Fujita, Y. (1973) Circulation Res. 33: 98–104

Urquilla, P. R., Marco, E. J., Lluch, S. (1975) Blood Vessels 12: 53–67