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Influence of calcium on noradrenaline release evoked by 5-hydroxytryptamine, tyramine and potassium from goat pial arteries

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The release of tritium (^3H) evoked by tyramine, potassium (K^+) and 5-hydroxytryptamine (5-HT) from goat pial arteries preloaded with [^3H]noradrenaline (^3H-NA) was studied. In normal Krebs-bicarbonate solution (KBS) all these agents caused a transient increase in radioactivity release over the basal spontaneous outflow. The pattern of release evoked by 5-HT was similar to that induced by tyramine with a slow onset and decline, but different from that induced by K^+ which produced a rapid peak of ^3H release followed by a quick fall. The removal of Ca^{2+} from the medium did not modify the efflux of radioactivity caused by tyramine, but the ^3H efflux produced by K^+ was markedly reduced. Nevertheless, in this Ca^{2+}-free medium the ^3H release evoked by 5-HT was partially, but significantly, decreased. These results indicate that K^+ evokes NA release by a Ca^{2+}-dependent process, probably of an exocytotic nature, while tyramine mediates NA release by means of a Ca^{2+}-independent mechanism. However, 5-HT possesses a Ca^{2+}-dependent and a tyramine-like component.

Tyramine releases noradrenaline (NA) from adrenergic nerves of peripheral tissues by means of a Ca^{2+}-independent process (Thoenen et al 1969; Chubb et al 1972; Fozard & Mwaluko 1976). Furthermore, it has been proved that potassium ions (K^+) release NA and dopamine-β-hydroxylase from sympathetic nerve endings by a Ca^{2+}-dependent process similar to the physiological efflux of adrenergic neurotransmitter (García & Kirpekar 1975; Thoa et al 1975).

On the other hand, it has been demonstrated that 5-hydroxytryptamine (5-HT) releases NA from several tissues (Fillion et al 1971; Fozard & Mwaluko 1976; Lluch et al 1976; McGrath 1977; Starke & Weitzell 1978; Marín et al 1979). However, this release is Ca^{2+}-dependent in the isolated rabbit heart (Fozard & Mwaluko 1976), while in the rabbit pulmonary artery it might be tyramine-like (Starke & Weitzell 1978).

In spite of these studies in peripheral tissues, little is known about the influence of Ca^{2+} on the NA evoked by these agents in brain vessels. Therefore we have made a comparative study of the influence of extracellular Ca^{2+} on the tritium [^3H]release elicited by tyramine, K^+ and 5-HT in goat pial vessels preloaded with ^3H-NA.

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MATERIALS AND METHODS

Preparation of tissues and ^3H efflux

Goats (20–40 kg) were killed by i.v. injection of 30 ml of saturated solution of KCl. The brain was removed and the arteries of the circle of Willis with their branches were dissected, placed in a Petri dish which contained Krebs-bicarbonate solution (KBS) and the blood immediately washed out. The arteries were then divided into two pools of similar weight (40–50 mg). Each pool was placed in a cylindrical nylon net and immersed in 4 ml of oxygenated KBS at 37 °C. After 15 min equilibration, tissues were exposed to $\pm^3$ H-NA (2×10^{-7}M, specific activity 10·7 Ci mmol^{-1}) for 30 min. One of the pools was washed with fresh KBS at 10 min intervals during 100 min while the other was transferred into Ca^{2+}-free KBS after 80 min of washout where it remained until the end of the experiment.

To measure the spontaneous ^3H release the arteries were successively immersed in 5 vials containing 2 ml of fresh KBS for 3 min periods. The drug-evoked release was analysed by transferring the tissue to another 4 vials, each one containing 2 ml of KBS with the appropriate concentration of the drugs studied (140 mM of K^+; 10^{-4} M of tyramine and 5-HT). Finally, the arteries were again exposed to fresh KBS in another 5 vials to allow a return to the basal level of ^3H efflux. The total radioactivity present in

the media was analysed by adding 0·5 ml of each sample to 10 ml of Bray's solution (Bray 1960) and measured in a Nuclear Chicago liquid Scintillation counter, model Isocap 300, using the external standard method to correct for quenching. The results are expressed as counts min^{-1} mg^{-1} of wet tissue.

Media

The composition of normal KBS was (mM): NaCl, 115; KCl, 4·6; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4.7H_2O$, 1·2; $NaHCO_3$, 25; glucose, 11·1 and the disodium salt of ethylenediaminetetraacetic acid (Na_2 EDTA) 0·03. This solution was equilibrated with 95% O_2 and 5% CO_2 and the final pH was 7·4–7·5. In the experiment with Ca^{2+}-free KBS, $CaCl_2$ was omitted. In some experiments, 1 mM of ethyleneglycol-bis (β-aminoethyl ether) *NN'*-tetraacetic acid (EGTA) was added to Ca^{2+}-free KBS.

Drugs, solutions and statistics

The drugs used were: tyramine hydrochloride (Sigma); 5-hydroxytryptamine creatinine sulphate (Sigma); potassium chloride (Merck); (±)-[^{3}H]-noradrenaline hydrochloride (Radiochemical Centre, Amersham).

Stock solutions of 5-HT and tyramine were prepared in 0·9% NaCl (saline) containing 0·01% (w/v) ascorbic acid and kept frozen (−20 °C). Stock solution of ^{3}H-NA was made in 0·01 HCl and stored at 4 °C.

High-K^+ solution (140 mM final in the vial) was prepared by adding the appropriate amount of KCl from a concentrated (saturated at room temperature) KCl to normal KBS. The effects of an equivalent hyperosmolar KBS, made up with sucrose, on ^{3}H efflux was also tested in 2 experiments.

Results shown in figures and the text were expressed as means ± s.e. Statistical analyses were performed using Student's *t*-test. A probability value of less than 5% was considered significant.

RESULTS

Spontaneous ^{3}H release

The spontaneous ^{3}H efflux from goat pial arteries preloaded with ^{3}H-NA showed a rapid initial decay followed by a moderate loss, which levelled off after approximately 90 min of washout.

Release of ^{3}H evoked by tyramine

Tyramine (10^{-4}M) increases the radioactivity release from brain vessels preincubated with ^{3}H-NA. The peak was reached after 6 min incubation with tyramine, then it slowly declined but remained over the basal release even 15 min after removal of the drug from the medium. Furthermore, in Ca^{2+}-free solution, with or without EGTA, the ^{3}H release induced by tyramine was unchanged (Fig. 1).

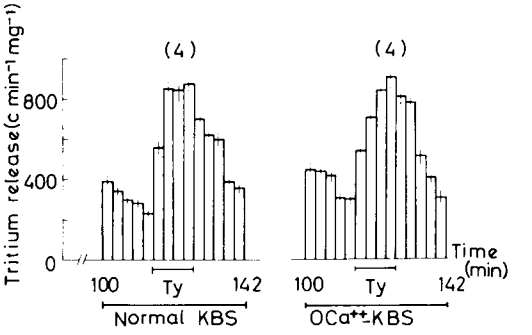

FIG. 1. Tritium release induced by tyramine (Ty, 10^{-4}M) from goat pial arteries in normal Krebs-bicarbonate solution (KBS) and KBS without Ca^{2+}. Tissues were previously labelled with ^{3}H-NA (2×10^{-7}M) and thoroughly washed during 100 min before the initiation of sample collection. Vials containing 2 ml of bathing solution were collected every 3 min. Each column represents the tritium efflux during a period of 3 min. Number of experiments are shown in parentheses. Vertical lines represent s.e. of the means. Horizontal lines indicate the time of exposure to different solutions.

^{3}H release elicited by high-K^+

Fig. 2 shows the marked increases of ^{3}H release from pial arteries evoked by 140 mM of K^+. The peak of radioactivity was obtained during the first 3 min and then quickly declined. When the arteries were incubated in a Ca^{2+}-free medium the release was greatly reduced ($P < 0.005$). The addition of sucrose to KBS to obtain a similar osmolarity to that of KCl solution, did not induce an increase of radioactivity over basal levels. This result indicates that the hypertonicity is not the cause of the release of ^{3}H induced by the high-K^+ solution.

Release of ^{3}H evoked by 5-HT

Fig. 3 shows the pattern of ^{3}H release induced by 5-HT (10^{-4}M). The incubation of the arteries with 5-HT produced a rise in the release of radioactivity which reached a peak 6 min later but remained over the basal release even 15 min after the drug was removed from the medium.

Ca^{2+}-deprivation, with or without EGTA, significantly diminished (50%) the peak of 5-HT-induced ^{3}H release ($P < 0.025$) with respect to the radioactivity evoked by this drug in normal KBS.

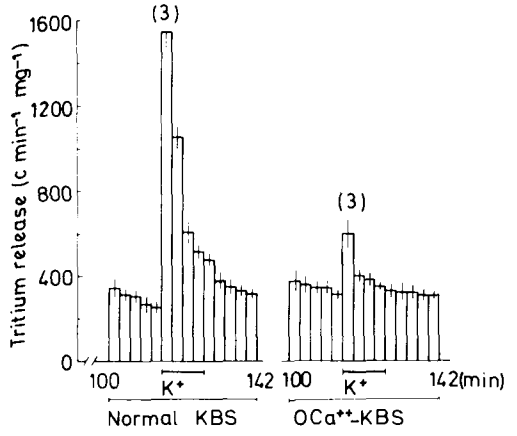

FIG. 2. Tritium release induced by potassium (K^+, 140 mM) from goat pial arteries in normal Krebs-bicarbonate solution (KBS) and Ca^{2+}-free KBS. Experimental design as in Fig. 1. Number of experiments are shown in parentheses. Vertical lines represent s.e. of the means. Horizontal lines indicate the time of exposure to different solutions.

DISCUSSION

The experiments described show that tyramine, 5-HT and K^+ induced ^3H release from goat cerebral arteries preloaded with ^3H-NA. The secretory effect of high K^+ concentrations was Ca^{2+}-dependent whereas with tyramine it was Ca^{2+}-independent. 5-HT was found to possess both Ca^{2+}-dependent and Ca^{2+}-independent components.

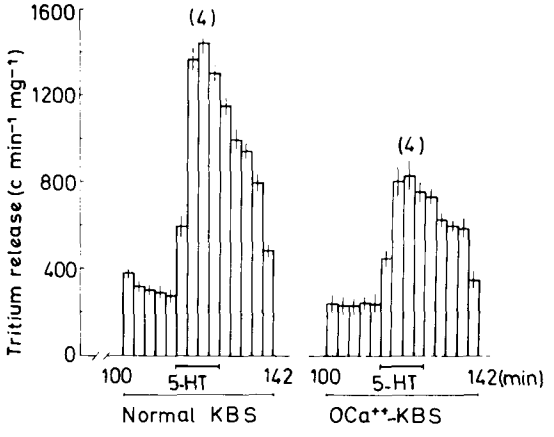

FIG. 3. Tritium release induced by 5-HT (10^{-4} M) from goat pial arteries in normal Krebs-bicarbonate solution (KBS) and Ca^{2+}-free KBS. Experimental design as in Fig. 1. Number of experiments are shown in parentheses. Vertical lines represent s.e. of the means. Horizontal lines indicate the time of exposure to different solutions.

The extracellular Ca^{2+} is one of the specific requirements for the release of NA from adrenergic nerves by physiological stimuli such as electrical stimulation or K^+ (Rubin 1970; Kirpekar 1975). Therefore, the fact that the NA release evoked by tyramine and K^+ was Ca^{2+}-independent and Ca^{2+}-dependent, respectively, suggests different mechanisms for their secretory effects. These differences are also found in peripheral tissues. Thus, it has been reported that K^+ depolarizes the adrenergic nerves causing NA-release by a Ca^{2+}-dependent exocytotic mechanism (Thoa et al 1975), while for tyramine this release is Ca^{2+} independent and of a non-exocytotic nature (Thoenen et al 1969; Chubb et al 1972; Thoa et al 1975; Garcia et al 1976).

On the other hand, our results show that tyramine and 5-HT present a similar pattern of ^3H-efflux, although the efflux evoked by 5-HT was greater than that caused by tyramine, when the same molar concentration was used. However, the influence of Ca^{2+} on the release was different depending on the agent used. Indeed, the release by tyramine was independent of extracellular Ca^{2+}, whereas for 5-HT it seemed to be partially dependent on this cation. These results are similar to those obtained in rabbit heart in which lowering the extracellular Ca^{2+} does not alter the ^3H efflux induced by tyramine but greatly reduces the ^3H release caused by 5-HT, although this was not completely suppressed (Fozard & Mwaluko 1976). However, it has been reported in rabbit pulmonary artery that 5-HT evoked NA-secretion might not be Ca^{2+}-dependent (Starke & Weitzell 1978). In addition, the application of 5-HT to the rabbit superior cervical ganglion neurons (Wallis & North 1978) or on the nerve fibres of rabbit cervical vagus (Riccioppo Neto 1978) induces a rapid depolarization. Therefore in summary, 5-HT evokes ^3H release from goat brain vessels by both a tyramine-like and a K^+-like mechanism, the latter could involve depolarization of perivascular adrenergic endings.

Acknowledgements

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REFERENCES

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

Chubb, I. W., De Potter, W. P., De Schaepdryver, A. F. (1972) Naunyn-Schmiedeberg's Arch. Pharmacol. 274: 281–286

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

Fozard, J. R., Mwaluko, G. M. P. (1976) Br. J. Pharmacol. 57: 115–125

García, A. G., Kirpekar, S. M. (1975) J. Pharmacol. Exp. Ther. 192: 343–350

García, A. G., Kirpekar, S. M., Sánchez-García, P. (1976) J. Physiol (London) 261: 301–317

Kirpekar, S. M. (1975) Prog. Neurobiol. 4: 163–210

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

Marín, J., Salaices, M., Sánchez, C. F. (1979) J. Pharm. Pharmacol. 31: 818–821

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

Riccioppo Neto, F. (1978) Eur. J. Pharmacol. 49: 351–356

Rubin, R. P. (1970) Pharmacol. Rev. 22: 389–428

Starke, K., Weitzell, R. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 304: 237–248

Thoa, N. B., Wooten, F. G., Axelrod, J., Kopin, I. J. (1975) Mol. Pharmacol. 11: 10–18

Thoenen, G. S., Huerliman, A., Haefely, W. (1969) Eur. J. Pharmacol. 6: 29–37

Wallis, D. I., North, R. A. (1978) Neuropharmacology 17: 1023–1028