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Release of noradrenaline from cat cerebral arteries by different drugs and potassium

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The existence of receptors for 5-hydroxytryptamine (5-HT, serotonin) which mediate the vasoconstrictor effects of this amine in the cerebral blood vessels, has been demonstrated [1–3]. Apart from this direct effect on the tryptaminergic receptors, it has been observed that 5-HT has the ability to release noradrenaline from several tissues [4–9], and this indirect effect contributes to the overall actions of 5-HT. Furthermore, Fozard and Mwaluko [6] have demonstrated on the isolated rabbit heart that this noradrenaline release is calcium-dependent, suggesting that it is an exocytotic-like process. However, Starke and Witzell [9] have reported that the greater effects of serotonin on the perivascular adrenergic nerve endings of the rabbit pulmonary artery seem to be tyramine-like.

The aim of the present investigation was to study in a comparative manner the mechanism of noradrenaline release evoked by serotonin from cat pial arteries with that induced by tyramine, ionophore X537A and potassium (K^+) whose patterns or mechanisms of adrenergic neurotransmitter release have been extensively studied in peripheral tissues. Thus, tyramine is taken up by the adrenergic nerve terminals and causes stoichiometric displacement and release of noradrenaline [10] by a non-exocytotic mechanism [11, 12]. Ionophore X537A is a drug which increases the permeability of membranes to calcium and other divalent and monovalent ions [13] causing secretion of catecholamines apparently by a non-exocytotic process [14–16]. Finally K^+ releases noradrenaline from adrenergic fibres by a process similar to the physiological efflux of adrenergic neurotransmitter, i.e. by exocytosis [17, 18].

Cats of either sex ranging in weight from 1.5 to 3 kg were anesthetized with 35 mg/kg of sodium pentobarbital administered intraperitoneally and killed by bleeding. The brain was carefully removed and the circle of Willis arteries with their ramifications were dissected and placed in a Petri dish which contained ice-cold Kreb–bicarbonate solution (KB). In this medium the blood was removed and the arteries (10–20 mg) pooled. Afterwards the arteries were placed on a cylindrical nylon net and put into a beaker containing 4 ml of oxygenated KB. After a 15 min equilibration period at 37°, the tissues were then exposed to (±) (^{3}H)-noradrenaline (^{3}H-NA, 2×10^{-7}M, specific activity 10.7 Ci/mmole) for 30 min and thoroughly washed with fresh KB solution at 10 min intervals during a 100 min period.

To estimate the spontaneous tritium release, the arteries were successively immersed in 5 vials containing 2 ml of fresh KB solution for 3 min periods. The drug-evoked release was analyzed by transferring the tissue to another 4 vials, each one containing 2 ml of KB with the appropriate concentration of the drugs studied (140 mM K^+; 10^{-5}M for all other drugs); finally the arteries were again exposed to fresh KB in another 5 vials in order to recover the basal level of tritium efflux. Total radioactivity present in the media was analyzed by adding 0.5 ml of each sample to 10 ml of Bray's solution [19] and it was 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 cpm/mg of wet tissue.

The composition of the normal KB was: NaCl, 119 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4.7H_2O$, 1.2 mM; $NaHCO_3$, 25 mM; glucose, 11.1 mM and disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), 0.03 mM. This solution was equilibrated with 95% O_2 and 5% CO_2 and the final pH was 7.4–7.5. In some experiments, 80 min after the incubation period, the arteries were washed with KB without calcium to the end of the experiment.

The following drugs were used: 5-hydroxytryptamine creatinine sulfate and tyramine hydrochloride (Sigma); potassium chloride (Merck); (±) (^{3}H)-noradrenaline hydrochloride (Radiochemical Centre, Amersham), and ionophore X537A (Ro2-2985, Lasalocid, Hoffman-La Roche).

Serotonin and tyramine were prepared as stock solutions in physiological saline containing 0.01% (w/v) ascorbic acid and kept frozen (−20°). Stock solutions of ^{3}H-NA were made in 0.01 N HCl and stored at 4°. X537A was dissolved in ethanol and stored at −20°; the final concentration of ethanol in the KB solution was always 0.1%.

High-K^+ solution (140 mM) was prepared by adding KCl saturated solution to KB to the adequate amount. In three experiments, the effects of and equivalent hyperosmolar Krebs solution (made up with sucrose, 252 mM) on ^{3}H-efflux were tested.

Results were expressed as means ± S.E. of the means. Deviations from means were statistically analyzed by the Student's *t*-test. A probability value of less than 5 per cent was considered significant.

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

Figure 1 shows the pattern of ^{3}H-release evoked by serotonin. Addition of 5-HT (10^{-5}M) to the incubation medium produced a rise of the release of radioactivity which reached the peak 6 min later, followed by a slow decrease.

Calcium-deprivation greatly inhibited the secretory effects of 5-HT. If the background release is subtracted from the drug-evoked release, the net efflux of tritium in the absence of calcium is significantly lower than in the presence of this cation ($P < 0.01$).

Tyramine (10^{-5}M) considerably increase the release of radioactivity from the brain arteries. This release was superior to that produced by the same concentration of 5-

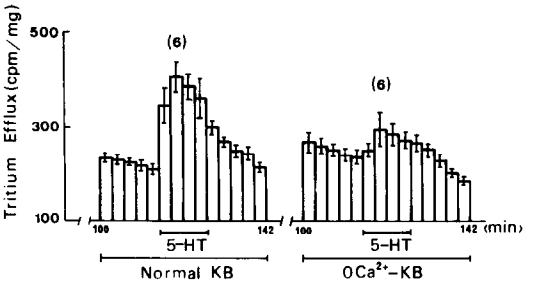

Fig. 1. Tritium efflux induced by 5-HT (10^{-5}M) from cat pial arteries in normal Krebs–bicarbonate solution (KB) and KB without Ca^{2+}. Tissues were previously labelled with ^{3}H-noradrenaline (2×10^{-7}M) and thoroughly washed during a 100 min period before the initiation of sample collection. Vials containing 2 ml 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.

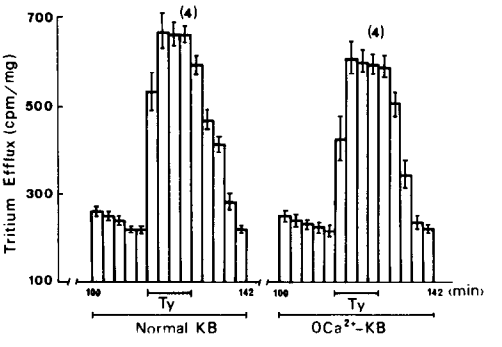

Fig. 2. Tritium efflux induced by tyramine (Ty, 10^{-5}M) from cat pial arteries in normal Krebs–bicarbonate solution (KB) and Ca^{2+}-free KB. Experimental design as in Fig. 1. Number of experiments are shown in parentheses. Vertical lines represent S.E. of the means. Horizontal lines indicate the time of exposure to different solutions.

HT in normal KB. The pattern of this efflux was similar to that obtained with serotonin. Furthermore, ^{3}H-release caused by tyramine was unchanged by removal of Ca^{2+} from the medium (Fig. 2).

Ionophore X537A (10^{-5}M) markedly increased the radioactivity efflux from cat pial arteries, approximately double of that obtained with tyramine. In addition, 0.1% ethanol did not have influence on the overall tritium efflux caused by X537A. The peak of the radioactivity released was reached during the first 6 min of incubation, declining later on, but remaining over the basal level in spite of removal of the drug from the medium. In O-Ca^{2+} solution, with or without EGTA (1 mM), the pattern of ^{3}H-release evoked by X537A was quite similar to that obtained in normal KB.

Exposure of pial arteries to K^+ (140 mM) induced a marked release of tritium which was similar to that obtained with X537A. The peak of release was reached in the first 3 min and then quickly declined. When the arteries were incubated in a Ca^{2+}-free medium the efflux was greatly reduced ($P < 0.05$). Hyperosmotic (252 mM sucrose) KB solution did not induce an increase of ^{3}H-efflux, indicating that the hypertonicity itself is not the cause of the release evoked by the hypertonic high-potassium solution.

The present experiments show that high K^+ concentrations, serotonin, tyramine and the ionophore X537A evoke tritium release from cat pial arteries whose endogenous NA stores had previously been labelled with tritiated NA. While the secretory response to K^+ and serotonin was dependent on extracellular Ca^{2+}, tyramine and the ionophore evoked large secretory responses even in the absence of this cation.

One of the specific requirements for the release of NA from adrenergic neurones by 'physiological stimuli' such as electrical stimulation or K^+ is its absolute requirement for extracellular Ca^{2+} [20, 21]. Consequently, the fact that the NA release induced either by serotonin or by high K^+ concentration was Ca^{2+}-dependent suggests that the mechanism of the secretory responses of both agents is similar. This same mechanism is also found in peripheral tissues. Thus, it has been demonstrated that K^+ and serotonin depolarizes the adrenergic nerves producing NA release by a Ca^{2+}-dependent exocytotic process [6, 18]. In addition, application of 5-HT to the rabbit superior cervical ganglion neurones [22] or to the nerve fibres of rabbit cervical vagus [23] induces a rapid depolarization. Therefore, serotonin-evoked release in cat pial arteries might be mediated by depolarizing the nerve terminals, facilitating Ca^{2+}-influx and subsequent transmitter secretion, although Starke and Weitzell [9] have recently reported that NA-release by serotonin from rabbit pulmonary artery does not seem to be clearly Ca^{2+}-dependent. A second possibility is that 5-HT could be directly opening Ca^{2+} channels in the adrenergic nerve terminals without depolarization, but this still has to be tested experimentally. Tyramine and X537A seem to induce tritium release by an entirely different mechanism, since their secretory responses are independent of extracellular Ca^{2+}. It is possible that both drugs could release the amine directly from the vesicles into the cytoplasm and then the transmitter would 'diffuse' away. This hypothesis is supported by the fact that these drugs do not induce a concomitant release of NA and dopamine beta-hydroxylase [12, 14, 18]. The Ca^{2+}-independence found for tyramine and the ionophore in brain vessels is also similar to that found in peripheral tissues [11, 12, 14, 15, 18].

Since these four procedures evoke the release of NA from cat cerebral arteries as well as from other peripheral adrenergic neuro-effector junctions [6, 11, 14, 15, 18] by similar mechanisms, we would like to take these findings as additional evidence supporting the presence of functional adrenergic nerve terminals innervating the brain vessels.

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Enhancement by transition metals of chromosome aberrations induced by isoniazid

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Isoniazid (isonicotinic acid hydrazide) is an effective antitubercular drug in widespread use [1, 2]. However, isoniazid has been found to induce tumors in mice [1] and chromosome aberrations *in vivo* [3] and *in vitro* [4]. At the same time there is little evidence for a carcinogenic or mutagenic effect in humans [1, 2, 5]. We have recently found that complexes of transition metals enhance unscheduled DNA synthesis induced by isoniazid [6] in cultured human cells. We report here that a manganese–glycine complex strongly enhances chromosome aberrations induced by isoniazid in Chinese hamster ovary (CHO) cells. A copper–glycine complex has a weak stimulatory effect, while iron–EDTA inhibits DNA damage by isoniazid. Chromosome aberrations induced by isoniazid or isoniazid/manganese mixtures are inhibited by simultaneous treatment with reduced glutathione or catalase. These results suggest that manganese may be important in the toxic and carcinogenic effects of isoniazid.

Procedures for the maintenance and preparation of CHO cells for chromosome aberration experiments, as well as harvesting and staining techniques, have been described [7]. Chemicals were obtained from the following sources: isoniazid (m.p. 169–171°), catalase (purified powder from bovine liver) and glutathione (reduced and oxidized forms) from the Sigma Chemical Co., St. Louis, MO; reagent grades of $CuSO_4 \cdot 5H_2O$, $FeCl_3 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, glycine, and EDTA from the Fisher Scientific Co., Vancouver, Canada. Metal complexes were prepared at 10^{-2} M in water [6] and diluted in medium [Eagle's minimum essential medium (MEM) plus 2.5% fetal calf serum]. Inactivated catalase was prepared by heating an aqueous solution of catalase (1 mg/ml) at 100° for 30 min. Freshly prepared solutions of chemicals in medium were mixed directly in the culture dishes. The order of addition was: glutathione, catalase, metal complex and isoniazid. All concentrations listed in Table 1 refer to final concentrations after mixing. Following a 3-hr treatment period, the cells were rinsed, incubated for 20 hr in growth medium (MEM plus 15% serum) and harvested [7]. In the analysis of chromosome aberrations only distinct breaks and exchanges were scored.

Elevated frequencies of chromosome aberrations were induced by treatment with high concentrations of isoniazid alone (Table 1). Up to 42 per cent of all metaphase figures contained aberrations, while the number of exchanges per metaphase reached a maximum of 2.1 at 2×10^{-2} M isoniazid.

The addition of a manganese–glycine complex at 10^{-5} M final concentration resulted in enhanced toxicity and high frequencies of chromosome aberrations at low concentrations of isoniazid. At 2×10^{-4} M isoniazid, the frequency of aberrations (53 per cent) and the number of exchanges per metaphase (2.5) exceeded the values obtained with 100-fold higher concentrations of isoniazid in the absence of manganese. The isoniazid/manganese mixtures were active at isoniazid concentrations similar to those measured in humans during treatment [2].

Relative to the strong effect of manganese, copper–glycine showed weak enhancement while iron–EDTA had none. There was approximately a 5-fold increase in the cytocidal and cytostatic effects of isoniazid when copper (10^{-4} M) was present. However, a higher frequency of chromosome aberrations was observed only at one concentration of isoniazid (5×10^{-3} M). At all other concentrations a similar level of aberrations was detected with or without added copper. In contrast, iron–EDTA at 10^{-4} M reduced the cytostatic and clastogenic effects of isoniazid.

Chromosome aberrations were not induced by the metal complexes at the concentrations employed in this study (Table 1). Treatment of cells with much higher (mM) concentrations of the metals induced aberrations, primarily chromatid breaks [7]. This is in contrast to the high frequencies of exchange observed in cells treated with isoni-