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The indirect sympathomimetic activity of etilefrine - a comparison with tyramine and ephedrine using [^{3}H]noradrenaline

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The indirect sympathomimetic activity of etilefrine has been examined using the ventral caudal artery of the rat. This vessel has a rich sympathetic innervation and lends itself to studies on [^{3}H]noradrenaline efflux from these sites. Etilefrine possessed significant indirect activity on the artery and this action, although less than that of tyramine, was equivalent to that caused by ephedrine. Pretreatment of the vessels with a mixture of iproniazid, doca, cocaine and UO521 (3′,4′-dihydroxy-2-methyl propiophenone) significantly enhanced[^{3}H]-noradrenaline efflux from the artery.

Etilefrine is a sympathomimetic amine that has been used in the treatment of orthostatic hypotension (Miller, Wiener & Bloomfield, 1973). In an earlier study (Frost, Frewin & Gerke, 1977) on blood vessels in a segment of the rat tail, it was suggested that the drug possessed both direct and indirect sympathomimetic components to its action.

We have further investigated the probability of etilefrine having indirect sympathomimetic activity and compared this component of its action with that of two other indirectly acting agents (tyramine and ephedrine). The ventral caudal artery of the rat was selected as the test vessel because of its rich sympathetic innervation (Hodge & Robinson, 1972) and because of the satisfactory responses obtained to etilefrine previously (Frost & others, 1977).

MATERIAL AND METHODS

Male Sprague-Dawley rats (250–350 g) were stunned and exsanguinated. The tail was then severed from the trunk and the ventral artery isolated. A piece of this artery measuring approximately 3 cm long was removed and placed in Krebs bicarbonate solution. The artery was blotted dry (with the intraluminal fluid being carefully expressed) and then weighed. The vessel was then preincubated for 30 min in Krebs bicarbonate solution with EDTA and ascorbic acid (As/K) at 37° and bubbled with 5% CO_2 in oxygen before being incubated in either (±)-[^{3}H]noradrenaline (^{3}H-NA) 1·18 μM or [^{14}C]sorbitol 1·1 μM for 30 min. The amount of radioactive material that effluxed from each treated artery at 1 min intervals when the vessel was washed with 1 ml aliquots of As/K solution in that time was recorded. This process continued for 20 min. Each 1 ml wash collected during this period was retained and added to 15 ml scintillation fluid which had the following composition (g litre^{-1}): PPO (2,5-diphenyloxazole) 8·25, POPOP (1,4-di [2-(5-phenyloxazolyl) benzene]) 0·25. The PPO and POPOP were dissolved in 1 litre of toluene to which was added 500 ml Triton X-100. The samples were counted on a Packard 2425 liquid scintillation spectrometer and corrected for efficiency using an automatic external standard. Results were expressed as p mol ^{3}H-NA g^{-1} min^{-1}.

The initial series of experiments examined the efflux profile of [^{14}C]sorbitol over 20 min from the rat tail artery, in the presence of (1) etilefrine (55·2 μM), (2) tyramine (1·45 mM), (3) cocaine (29·5 μM) and (4) deoxycorticosterone acetate (doca) (26·9 μM) with UO521 (55·0 μM). A control series of efflux studies using labelled sorbitol was also carried out in the absence of any drugs.

The subsequent experiments examined the efflux of ^{3}H-NA both in the presence and absence of the agents listed. In addition, the effect of (a) ephedrine (182 μM), (b) etilefrine (55·2 μM) with cocaine (29·5 μM) and (c) tyramine (1·45 mM) with cocaine (29·5 μM) was also examined. On the basis of results obtained during the series with sorbitol, the experimental protocol was modified for the ^{3}H-NA study. The modification consisted of an initial 5 min wash in As/K solution followed by a 15 min wash in As/K containing the individual drug(s).

A further series was carried out using arteries that were variously treated. These included: (1) pretreatment of rats with iproniazid (200 mg kg^{-1}, i.p. each day for 2 days) and exposing the segments of

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the tail arteries during the washout period to As/K containing cocaine (29·5 μM), doca (26·9 μM), and UO521 (55·0 μM); (2) pretreating rats with guanethidine (25 mg kg^{-1}, i.p.) on alternate days for 7 weeks, before removing their caudal arteries.

The content of compounds with a dihydroxyphenol structure in the efflux was calculated by combining five 1 min (i.e 1 ml) samples and subjecting each to a batch alumina procedure (de la Lande, Glover & Head, 1967) to determine what percentage of the total radioactivity was adsorbed onto the alumina.

Mean efflux curves were calculated from the results of at least five individual experiments.

Drugs used

[^{14}C]Sorbitol (10 mCi mmol^{-1}) (Amersham); (±)-[^{3}H]noradrenaline hydrochloride (10·8–12·0 Ci mmol^{-1}) (Amersham) purified by batch alumina process (Head, Crabb & others, 1977); etilefrine hydrochloride (Effortil, Boehringer Ingelheim); ephedrine hydrochloride (Knoll); tyramine hydrochloride (Koch-Light); UO521 (3′,4′-dihydroxy-2-methyl propiophenone) (Upjohn); cocaine hydrochloride (Macfarlan-Smith); deoxycorticosterone acetate (Steraloids); iproniazid (Marsilid, Roche); guanethidine sulphate (Ismelin, Ciba).

RESULTS

[^{14}C]Sorbitol. Efflux from extracellular space

The results of experiments in which the [^{14}C]sorbitol efflux was measured indicated that the counts obtained after 5 min efflux had decreased to approximately 5% of the counts effluxed at the first minute. For this reason, the efflux of ^{3}H-NA from the artery during the first 5 min was discarded, to avoid complicating the overall results with those due to this marked efflux from the extracellular space. The rate of efflux of [^{14}C]sorbitol was unchanged by the addition of any of the drugs used.

^{3}H-NA efflux. Control study

It was found that 90% of the total radioactivity was adsorbed onto alumina indicating that the collected efflux was predominantly dihydroxyphenol structured compounds—probably ^{3}H-NA or ^{3}H-DOPEG which are both of neuronal origin.

^{3}H-NA efflux in the presence of sympathomimetic amines

The comparative rates of efflux in arteries treated with etilefrine, tyramine and ephedrine are also shown in Fig. 1. The rate of efflux was in the descending order tyramine > ephedrine ≡ etilefrine > control. In the presence of cocaine, no significant difference was seen between the rates of efflux for cocaine alone and cocaine and etilefrine (Fig. 2a). The efflux rate for tyramine in the presence of cocaine was significantly greater than the efflux curve obtained for cocaine alone, irrespective of whether tyramine was introduced at min 5 (Fig. 2a) or at min 10 (Fig. 2b).

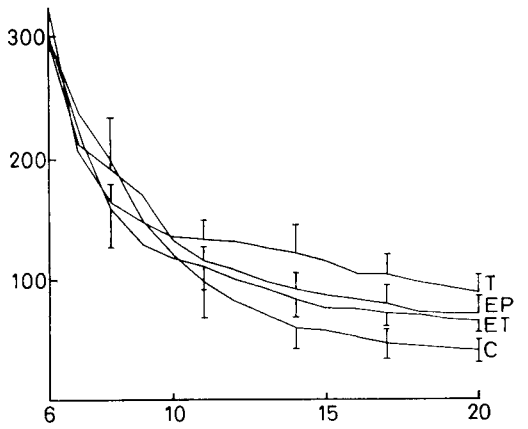

FIG. 1. Efflux curves of ^{3}H-NA from control (C) arteries and arteries treated with tyramine (T), ephedrine (EP) and etilefrine (ET). Each point represents the mean values ± s.e.m. of five experiments. Ordinate: Rate of efflux (p mol g^{-1} min^{-1}). Abscissa: Time (min).

^{3}H-NA efflux from pretreated arteries

The efflux curves from arteries which had been pretreated with guanethidine are shown in Fig. 3. It can be seen that although the amount of ^{3}H-NA effluxed is lower than the values in the control efflux curves (Fig. 1), etilefrine has caused an enhanced efflux of ^{3}H-NA compared with the values obtained when the vessels were not treated with etilefrine.

In Fig. 4 the efflux curves from arteries treated with iproniazid, cocaine, doca and UO521 are depicted. The arteries treated with etilefrine show an enhanced rate of efflux in relation to the untreated control arteries (Fig. 1).

DISCUSSION

The overall results of the present study support the earlier findings (Frost & others, 1977) that etilefrine possesses a significant indirect sympathomimetic component. The results show that etilefrine enhanced the rate of efflux of ^{3}H-NA from the sympathetically innervated rat tail artery, in comparison to control values. The rate of efflux was less than that induced by tyramine but comparable with that of ephedrine.

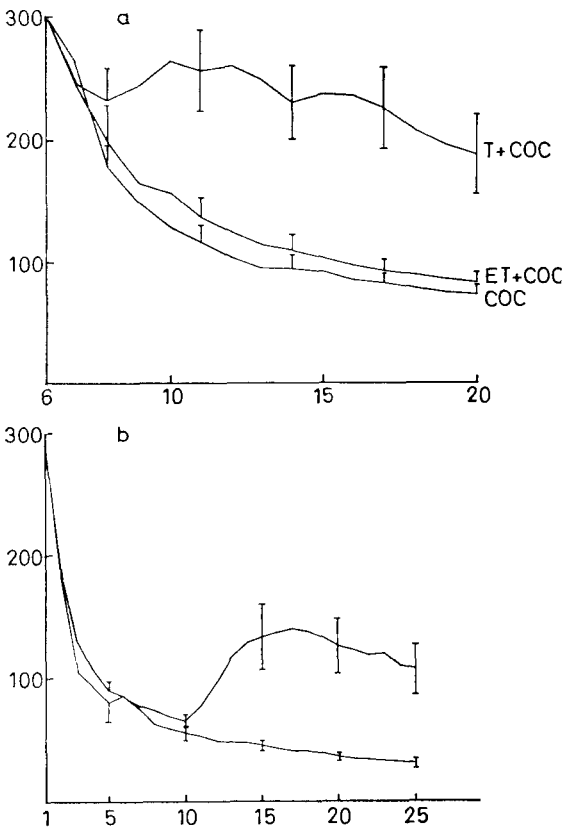

FIG. 2(a). Efflux curves of ^{3}H-NA from arteries treated with cocaine (COC), etilefrine and cocaine (ET + COC), and tyramine and cocaine (T + COC). Each point represents the mean values ± s.e.m. of five experiments. The cocaine and tyramine were administered together at min 5. In Fig. 2(b) cocaine was administered 5 min before tyramine (which was added at 10 min). Ordinate: Rate of efflux (p mol g^{-1} min^{-1}). Abscissa: Time (min).

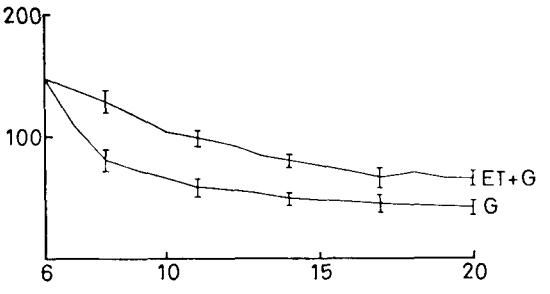

FIG. 3. Efflux curves of ^{3}H-NA from arteries obtained from rats chronically treated with guanethidine (G), or those pre-treated with guanethidine to which etilefrine was added (ET + G). Each point represents the mean values ± s.e.m. of five experiments. Ordinate: Rate of efflux (p mol g^{-1} min^{-1}). Abscissa: Time (min).

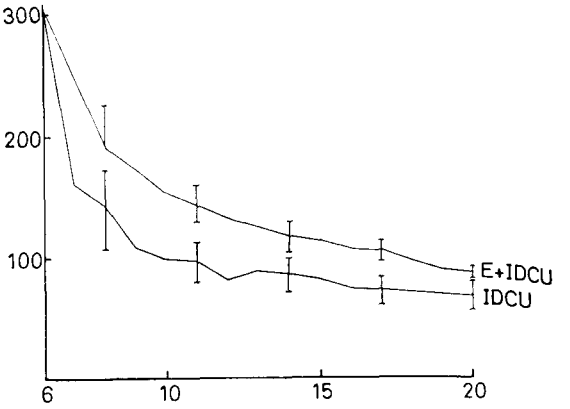

FIG. 4. Efflux curves of ^{3}H-NA from arteries treated with iproniazid, to which doca, cocaine and UO521 were added (IDCU) or from those to which etilefrine was further added (E + IDCU). Each point represents the mean values ± s.e.m. of five experiments. Ordinate: Rate of efflux (p mol g^{-1} min^{-1}). Abscissa: Time (min).

The predominant component of tyramine's sympathomimetic action relates to noradrenaline release from sympathetic nerve endings (Burn & Rand, 1958). In the present study, tyramine produced the greatest efflux of the three agents tested, while ephedrine, which has both direct and indirect effects (Burn, 1932), produced an efflux rate comparable with etilefrine. Previous evidence (Tainter & Chang, 1927; Tainter, 1929) showed that the actions of tyramine and ephedrine were antagonized by cocaine. In the present study the efflux of ^{3}H-NA from tyramine-treated arteries in the presence of cocaine was very significantly augmented. This augmentation occurred when cocaine and tyramine were added together to the wash fluid and also when tyramine was added 5 min after the application of cocaine. In the latter series the rate of efflux of ^{3}H-NA was relatively stable after 10 min washing, when tyramine was added. With etilefrine, the increase of ^{3}H-NA efflux was not significantly different from the values obtained with cocaine alone (Fig. 2a).

The depressed rate of efflux obtained following guanethidine pretreatment indicates that the storage potential of ^{3}H-NA has been decreased (presumably intraneuronally). Evidence for this phenomenon comes from the work of Bisson & Muscholl (1962) and Schanker & Morrison (1965) who showed that guanethidine is selectively accumulated in sympathetic nerves, thus affecting noradrenaline storage. Etilefrine's action in the guanethidine treated arteries which had been pre-incubated with ^{3}H-NA appeared unimpaired. The difference in efflux rate when this

drug was added was apparent over virtually the entire washout period.

Arteries treated with iproniazid, cocaine, doca and UO521 simultaneously had efflux rates higher than the control values. This was expected since cocaine and doca block neuronal and extraneuronal uptake, respectively, leading to increased loss of ^{3}H-NA from the arteries. In addition, iproniazid and UO521 would tend to decrease the breakdown of ^{3}H-NA, thereby providing more ^{3}H-NA to be available for release from the artery.

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