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Possible mechanism of acetaldehyde-induced noradrenaline release from sympathetic nerve terminals in isolated blood vessels.

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1 Vasoconstrictor responses to acetaldehyde were investigated in isolated and perfused canine intermediate auricular (ear) arteries.

2 Single injections of small doses of acetaldehyde (1–3 μmol) induced vasoconstriction in a dose-related manner and showed no tachyphylaxis. On the other hand, large doses of acetaldehyde (10–30 μmol) frequently caused tachyphylaxis when injected at 10 min intervals.

3 After tyramine treatment, constrictions induced by a large dose of acetaldehyde were consistently restored temporarily.

4 The acetaldehyde-induced vasoconstriction was inhibited by bunazosin, a potent α_1-adrenoceptor antagonist.

5 A small dose of imipramine blocked tyramine-induced vasoconstriction, but had no significant influence on noradrenaline (NA)-induced constrictions, and caused slight potentiation of acetaldehyde-induced constrictions.

6 Hydrocortisone treatment did not modify tyramine-induced vasoconstrictions and slightly suppressed NA- and acetaldehyde-induced constrictions but not significantly.

7 It is suggested that acetaldehyde causes a release of NA from a NA store of the sympathetic nerve terminals which is different from the tyramine-sensitive NA store, and that the acetaldehyde-sensitive NA store may be readily filled up with NA from the tyramine-sensitive store.

Introduction

Nelson (1943) showed that acetaldehyde had sympathomimetic activity in dogs in which the adrenal veins were tied. Akabane *et al.* (1964, 1965) also reported that acetaldehyde exerted its action through adrenergic mechanisms. It is well known that acetaldehyde releases catecholamines from sympathetic nerve terminals of the blood vessel, as reviewed by Vanhoutte *et al.* (1981). In 1968, Bell reported that tetrodotoxin (TTX) did not prevent the release of noradrenaline (NA) by tyramine. In 1975, Lai & Hudgins found that TTX abolished the response of rat vas deferens to transmural stimulation, but responses to acetaldehyde and tyramine were not impaired. However, acetaldehyde-induced effects were not inhibited by an uptake blocker, cocaine which blocked tyramine-induced effects (Lai & Hudgins, 1975). Thus, it was suggested that tyramine utilizes the neuronal uptake mechanism, but acetaldehyde appears to diffuse across the membrane by virtue of its lipid solubility in order to gain access to the same tissue transmitter storage sites. More recently, it was demonstrated that repetitive administration of acetaldehyde readily caused tachyphylaxis, but, after tyramine treatment, acetaldehyde-induced vasoconstriction was temporarily restored in isolated and perfused mesenteric arteries (Chiba & Tsukada, 1987), suggesting that tyramine may induce a release of NA mostly from the vesicle to the neuronal cytosol, and acetaldehyde may cause a release of NA from the cytosol to the extracellular space.

In the present study, we attempted to confirm the difference in the NA releasing actions between acetaldehyde and tyramine, by use of the cannula inserting method (Hongo & Chiba, 1983; Tsuji & Chiba, 1984) and to clarify the nature of the NA stores released by the two compounds, by use of isolated and perfused canine intermediate auricular (ear) arteries because the dog intermediate auricular

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arteries are extremely sensitive to an indirect NA releasing agent like tyramine (Ito & Chiba, 1985; Chiba & Ito, 1985).

Methods

Twenty-one mongrel dogs weighing from 8 to 18 kg were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). After treatment with sodium heparin (200 units kg^{-1}, i.v.), the animals were killed by rapid exsanguination from the right common carotid artery. The intermediate auricular artery of either ear was then carefully isolated, and 1–3 segments (without large branches, 0.4–0.7 mm in outer diameter, 4–7 mm in length) were cut from each isolated artery. A stainless-steel cannula (27 gauge, 0.4 mm outer diameter and 3 cm in length) with one small hole at a distance of 3 mm from the distal blind end was inserted into the arterial segment which was tied to the cannula distal to the hole, and thus the stream from the hole of the cannula passed only through the endothelial surface of the vascular segment as reported previously (Tsuji & Chiba, 1984). The cannulation procedure was performed in cold Krebs solution at a temperature of 4–10°C. We were careful to avoid endothelial damage, although it is possible that the insertion of the cannula may have caused some functional disturbance of the endothelium. The stainless steel cannula with the arterial preparation attached was placed in a cup-shaped glass container and perfused with constant flow by means of a peristaltic pump (Harvard Apparatus, Model 505-1210). The perfusate contained (mmol l^{-1}): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25 and glucose 5.6. It was bubbled with a mixture of 95% O_2 and 5% CO_2 to maintain the pH at 7.2–7.4 and was maintained at a constant temperature of 37°C. The glass container was also warmed with a circular pump (Haake) at a constant temperature of 37°C. The flow rate (1–2 ml min^{-1}) was adjusted at the beginning of the experiment to obtain a basal perfusion pressure of approximately 100 mmHg. The perfusion pressure was measured with an electronic manometer (Nihon Kohden, RP 2), and a vasoconstrictor response was recorded as an increase in perfusion pressure. Before the experiments were started, the preparations were allowed to equilibrate for over 1 h in the bathing medium. The original method for inserting cannula has been described in previous papers (Hongo & Chiba, 1983; Tsuji & Chiba, 1984; Ito & Chiba, 1984).

Drugs used in this study were acetaldehyde (Wako), tyramine hydrochloride (Tokyo Kasei), noradrenaline hydrochloride (NA, Sankyo), bunazosin hydrochloride (Eisai), imipramine hydrochloride (Fujisawa), and hydrocortisone sodium phosphate (Banyu).

The drug solution was administered into the rubber tubing close to the cannula in a volume of 0.01 to 0.03 ml for a period of 4 s.

The data are presented as mean ± s.e.mean in the text and illustrations. Student's t test was used and a P value of 0.05 or less was considered significant.

Results

Effects of intraluminal injection of acetaldehyde to isolated and perfused intermediate auricular arteries

When acetaldehyde was administered intraluminally to the arterial preparation, an increase in perfusion pressure was induced. A relatively small dose of acetaldehyde (0.1–3 μmol) caused dose-dependent vasoconstrictions with no tachyphylaxis. However, a large dose of acetaldehyde (10–30 μmol) readily caused tachyphylaxis. As shown in Figure 1, 1–3 μmol of acetaldehyde induced the same degree of repetitive vasoconstrictions in 10 min intervals, but 10–30 μmol of acetaldehyde clearly induced tachyphylaxis.

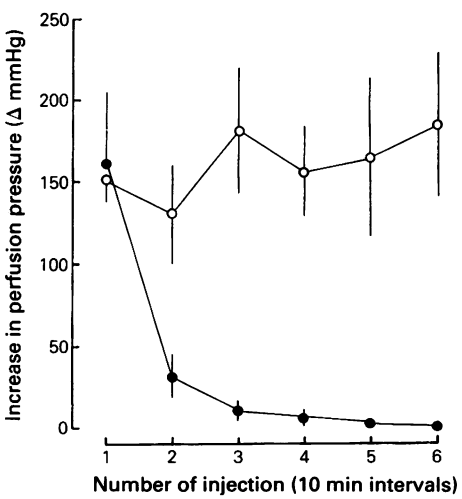

Figure 1 Vascular responses to repeated injections of small doses (○, 1–3 μmol) and large doses (●, 10–30 μmol) of acetaldehyde at 10 min intervals in isolated and perfused dog intermediate auricular arteries. Each point shows the mean value, and each vertical bar shows the standard error. $n = 5$ for both small and large doses.

Effects of tyramine treatment on acetaldehyde-induced tachyphylaxis

Tyramine readily induced vasoconstrictions in these ear arterial preparations as reported previously (Chiba & Ito, 1985). After establishment of acetaldehyde tachyphylaxis, effects of tyramine on the tachyphylaxis were examined. An injection of acetaldehyde was made after cessation of tyramine-induced vasoconstriction. After the disappearance of vasoconstriction to repeated injections of a large dose of acetaldehyde (30 μmol), a single injection of tyramine (10 and 100 μg) caused a temporary restoration of acetaldehyde-induced vasoconstriction as shown in Figure 2.

Effects of benazosin on acetaldehyde-, NA- and tyramine-induced vasoconstrictions

When NA or tyramine was administered, vasoconstrictor responses were dose-dependently induced as reported previously (Ito & Chiba, 1985; Chiba & Ito, 1985). A relatively small dose of acetaldehyde induced a vasoconstriction in a dose-related manner. The vasoconstrictor responses to these 3 compounds were significantly inhibited by 10 μg of bunazosin, a potent α_1-adrenoceptor antagonist (Shoji, 1981). Since the blocking effect of a single injection of 10 μg of bunazosin on noradrenaline-induced vasoconstrictions persisted for 20–30 min, each agonist was administered within 20 min in a series of experiments. Summarized data are shown in Figure 3.

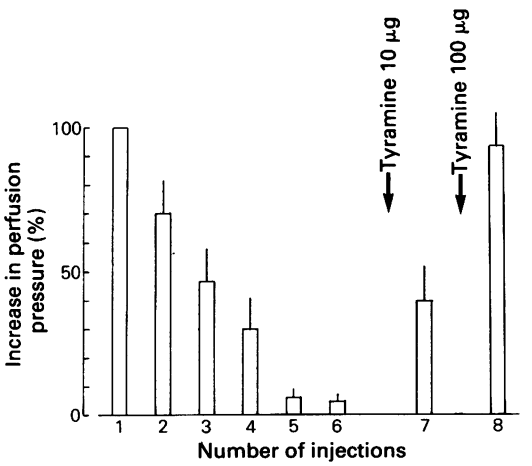

Figure 2 Summarized data of development of tachyphylaxis in responses to repetitive injections of 30 μmol of acetaldehyde and recovery after treatment with 10 and 100 μg of tyramine. Initial injection of 30 μmol of acetaldehyde induced an increase of 191 ± 43 mmHg (mean ± s.e.mean) in perfusion pressure in 6 preparations. Vertical bars shows s.e.means.

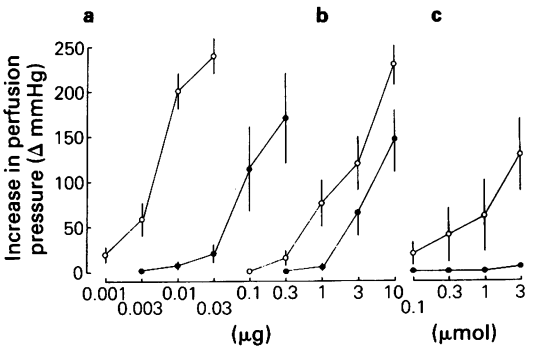

Figure 3 Blocking effects of 10 μg of bunazosin on (a) noradrenaline-, (b) tyramine- and (c) acetaldehyde-induced vasoconstrictions in dog isolated intermediate auricular arteries: (○) control; (●) after 10 μg bunazosin. Values represent the means with s.e.mean shown by vertical bars; n = 4–9.

Effects of imipramine on acetaldehyde-, NA- and tyramine-induced vasoconstrictions

When imipramine was injected in doses of 1 or 10 μg, it produced no significant vascular response. After treatment with 1 μg of imipramine, NA-induced constrictions were not significantly modified, but tyramine-induced constrictions were significantly suppressed as reported previously (Chiba & Ito, 1985). On the other hand, acetaldehyde-induced constrictions were significantly potentiated. Figure 4 shows the summarized data. After treatment with a

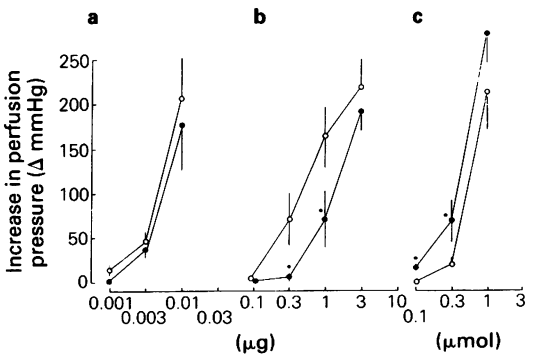

Figure 4 Effects of 1 μg of imipramine on (a) noradrenaline-, (b) tyramine- and (c) acetaldehyde-induced vasoconstrictions in dog isolated intermediate auricular arteries: (○) control; (●) after 1 μg imipramine. Values represent the mean with s.e.mean shown by vertical bars; n = 5–9; * $P < 0.05$; ** $P < 0.01$.

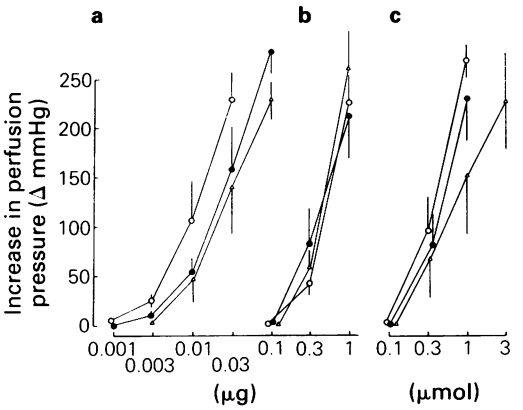

Figure 5 Effect of 10 and 100 μg of hydrocortisone on (a) noradrenaline-, (b) tyramine- and (c) acetaldehyde-induced vasoconstrictions in dog isolated intermediate auricular arteries: (○) control; (●) after 10 μg hydrocortisone; (△) after 100 μg hydrocortisone. Values represent the mean with s.e.mean shown by vertical bars; $n = 5$–7.

relatively large dose of 10 μg of imipramine, NA- and acetaldehyde-induced vasoconstrictions were also suppressed (data not shown).

Effects of hydrocortisone on NA-, tyramine- and acetaldehyde-induced vasoconstrictions

Hydrocortisone in doses of 1–100 μg did not produce any significant vascular response. NA- and acetaldehyde-induced vasoconstrictions were not significantly suppressed by 10 and 100 μg of hydrocortisone. Tyramine-induced vasoconstrictions were not modified by 10 and 100 μg of hydrocortisone. Summarized data are shown in Figure 5.

Discussion

It has been recognized that tyramine acts indirectly, following uptake into the adrenergic nerve terminal, by stoichiometric displacement of NA from storage sites in the cell (Furchgott *et al.*, 1963; Carlsson & Waldeck, 1965), and it is considered that the vesicle is the immediate source of the NA released (Smith, 1973). It is known that NA is displaced by tyramine or amphetamine in a cytoplasmic mobile pool I (Koelle, 1975). In the present study, tyramine-induced vasoconstrictions were significantly inhibited by pretreatment of the arterial preparation with imipramine, an $Uptake_1$ blocking agent but not by hydrocortisone, an $Uptake_2$ blocking agent. A large dose of hydrocortisone suppressed both acetaldehyde- and NA-induced vasoconstrictions, indicating its postsynaptic depressant action. Thus, in this study $Uptake_2$ mechanisms (Iversen, 1965) may not be involved for acetaldehyde-induced actions. Acetaldehyde-induced vasoconstrictions were inhibited by an α_1-adrenoceptor antagonist, bunazosin, as well as tyramine-induced ones, but they were rather enhanced after imipramine treatment, indicating the different NA releasing action of acetaldehyde from that of tyramine.

Eade (1959) reported that the sympathomimetic action of acetaldehyde was inhibited in reserpine-treated cats. He also showed that cocaine potentiates the aldehyde sympathomimetic responses but depresses the responses to tyramine. As reviewed by Vanhoutte *et al.* (1981), the NA stored in the adrenergic varicosity might be released by three mechanisms: (1) leakage induced, for example by organic solvents, (2) pharmacological displacement as by indirect sympathomimetics, and (3) exocytotic release by membrane depolarization. Several compounds containing acetaldehyde increase the permeability of the storage vesicles and favour the

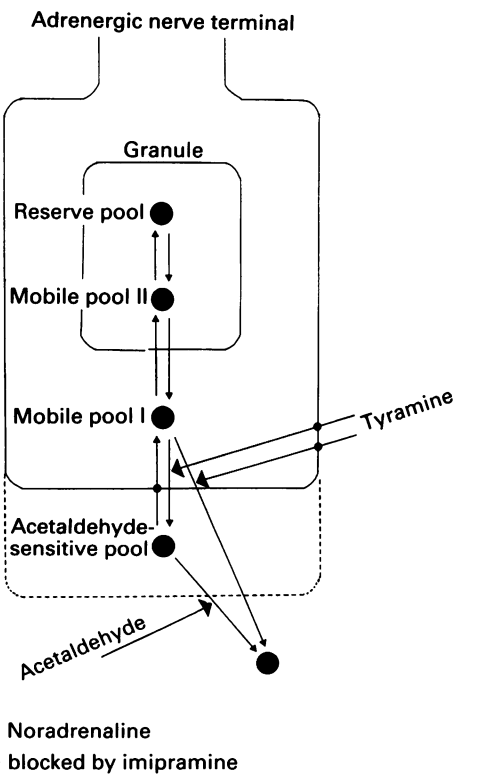

Figure 6 A schematic representation of acetaldehyde- and tyramine-sensitive noradrenaline (NA) pools in the adrenergic nerve ending. Mobile pool I; tyramine-sensitive store.

leakage of stored transmitter. Tyramine is a representative compound causing pharmacological displacement. In this study, we considered that acetaldehyde may cause release of NA from the acetaldehyde-sensitive NA pool by leakage but this pool is different from the tyramine-sensitive NA pool (mobile cytosol pool I).

Kobayashi *et al.* (1979) reported that acetaldehyde induced positive chronotropic and inotropic responses by releasing catecholamines from the adrenergic nerve stores in isolated canine atrial preparations. They also reported that the acetaldehyde-induced effects were inhibited by β-adrenoceptor blockade, but not by tetrodotoxin. Furthermore, imipramine frequently caused an enhancement of acetaldehyde-induced cardiac actions. In this study, we confirmed that acetaldehyde induced a marked vasoconstrictor response which was blocked by α-adrenoceptor blockade. In a previous paper (Chiba & Tsukada, 1987), we briefly reported that acetaldehyde produced a marked vasoconstriction but readily caused tachyphylaxis in isolated and perfused canine mesenteric arteries, making it very difficult to analyse pharmacological properties. However, in this study on the isolated ear arteries of the dog, relatively small doses of acetaldehyde induced a repetitive vasoconstriction without tachyphylaxis. As reported before (Chiba & Ito, 1985), in the isolated intermediate auricular arteries, an indirect sympathomimetic substance, tyramine usually produced marked vasoconstriction but in the isolated mesenteric arteries only a small vasoconstriction was induced even by large doses of tyramine. Thus, it is assumed that the intermediate auricular arteries are useful vessels for the investigation of NA releasing mechanism in the adrenergic nerve terminals. The NA in the granule may be provided for the neuronal cytosol. A large amount of acetaldehyde readily caused tachyphylaxis probably because the acetaldehyde-sensitive NA pool might not be readily filled up, and it is well known that tyramine causes a release of NA from the tyramine-sensitive NA store (mobile pool I). It is considered that NA released by tyramine may come out not only to the extracellular space but also to the neuronal cytosol (acetaldehyde-sensitive pool). Thus, after treatment with tyramine, acetaldehyde-induced vasoconstriction might be restored temporarily by the increasing cytosol NA in the acetaldehyde-sensitive store. As shown in Figure 6, acetaldehyde may cause a release of NA from the cytosol in the acetaldehyde-sensitive pool but not from the tyramine-sensitive pool (mobile pool I). Imipramine may have uptake blocking properties not only from the extraneuronal site to the intraneuronal vesicle but also from the intraneuronal acetaldehyde-sensitive pool to the tyramine-sensitive pool, because the acetaldehyde-induced vasoconstriction was slightly enhanced by imipramine treatment, although the tyramine-induced vasoconstriction was significantly inhibited.

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