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EFFECT OF TUAMINE, HEPTAMINOL AND TWO ANALOGUES ON UPTAKE AND RELEASE OF CATECHOLAMINES IN CULTURED CHROMAFFIN CELLS

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Abstract—The effects of tuamine (1-methylhexylamine), a sympathomimetic compound with hypertensive properties, heptaminol (6-amino-2-methyl-2-heptanol), an aliphatic amine with pressor properties, and two structural analogues of tuamine on high-affinity Na^+-dependent noradrenaline uptake and on nicotine-evoked release were examined in bovine chromaffin cells maintained in primary culture for 3 to 6 days. Tuamine was found to be a potent competitive inhibitor of noradrenaline uptake with an effect similar to that of cocaine. Its inhibition constant (K_i) was $1.1 \pm 0.1\ \mu M$ while K_i values of heptaminol, of 1-methylamino-5-pentanol oxalate and of 5-amino-2-methylhexanol oxalate, which were also found to be competitive inhibitors of noradrenaline uptake, were $60 \pm 2\ \mu M$, $260 \pm 28\ \mu M$ and $48 \pm 76\ \mu M$, respectively. Tuamine, heptaminol and 5-amino-2-methyl-2-hexanol were also shown to be inhibitors of nicotine-induced release of catecholamines, with IC_{50} values of $26 \pm 2\ \mu M$, $650 \pm 11\ \mu M$ and $500 \pm 10\ \mu M$, respectively. Tuamine and heptaminol did not inhibit noradrenaline release evoked by 59 mM K^+, suggesting that it acts at a step prior to calcium entry. The pharmacological properties of heptaminol as an anti-hypotension agent may partially account for its inhibitory effect on catecholamine uptake and release.

Chromaffin cells in adrenal medulla are derived from the neural crest [1]. As for sympathetic neurones, they have the capacity to synthesize neurotransmitter molecules, noradrenaline and adrenaline, to store them in secretory granules, and to release them on stimulation of receptors with acetylcholine [2, 3]. After the exocytotic event, chromaffin cells are also capable of taking up exogenous catecholamines by a well-characterized specific sodium-dependent transport process localized at the plasma membrane level [4, 5].

It has been established that noradrenaline and its metabolically related analogues, dopamine and adrenaline, are transported into chromaffin cells from two different types of sites on the plasma membrane: one is sensitive to cocaine, has a high affinity for noradrenaline, is strictly dependent on sodium and is saturating, while the other is insensitive to cocaine, is not saturating, has a low affinity to noradrenaline and is not dependent on sodium [5, 6].

Drugs blocking the catecholamine transport system have been widely studied [4, 7, 8] and many are used as therapeutic agents. Pharmacological studies have shown that heptaminol (6-amino-2-methyl-2-heptanol), and aliphatic amine with an alcoholic group on carbon 2, causes long lasting increases in blood pressure [9–11] accompanied by tachycardia and contraction of nictitating membrane in cat [12]; in rat, the pressure effects are less pronounced [12].

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Tuamine (1-methylhexylamine), a primary amine which is structurally related to heptaminol, is used as a sympathomimetic drug [13, 14]. Because the effects of heptaminol have been found to be abolished in cocaine-treated animals and in reserpine-treated animals, it has been suggested that this drug exerts its action by interfering with release and uptake of catecholamines [12]. In the present study we have investigated and further characterized the effects of tuamine, heptaminol and two analogues of tuamine on noradrenaline uptake and release from cultured bovine chromaffin cells, a system which has proven a suitable model for such studies [5, 15].

MATERIALS AND METHODS

Materials. Collagenase (EC 3.4.24.3) was purchased from Boehringer (Mannheim, F.R.G.), Percoll from Pharmacia (Bois d'Arcy, France), Dulbecco's modified Eagle's medium and fetal calf serum from GIBCO BRL (Cergy-Pontoise, France), [7-^{3}H]noradrenaline chloride (14 Ci/mmol) from Amersham (Les Ulis, France). Cytosine arabinoside and fluorodeoxyuridine were from Aldrich Chimie (Strasbourg, France). Tuamine, heptaminol, 1-methylamino-5-pentanol oxalate and 5-amino-2-methylhexanol oxalate were products from Centre de Recherche Delalande (Rueil-Malmaison, France). All other reagents were from Merck (Darmstadt, F.R.G.) or the Sigma Chemical Co. (St Louis, MO, U.S.A.).

Isolation and culture of bovine chromaffin cells.

Primary collagenase-dissociated chromaffin cells from bovine adrenal medulla were prepared on self-generating Percoll gradient as previously described [16] and maintained in culture in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum containing 10 μM cytosine arabinoside and 10 μM 5-fluorodeoxyuridine. Cells were grown on 24 multiple 16-mm well Costar plates (Costar, Data Packaging Corp., Cambridge, MA, U.S.A.) at 37° in a humidified 5% CO_2/95% air atmosphere at a cell density of 250,000 cells/well and used within 3–6 days after plating.

Uptake studies. Cultured cells were first incubated at 37° for 50 min in 1 mL fresh DMEM* free of antimitotics and amino acids; the medium was then removed and cells were incubated for 10 min in the presence of tuamine, heptaminol or analogues at the indicated concentrations in 1 mL DMEM. Cells were then incubated for the indicated times with 1 μCi [7-^{3}H]noradrenaline in 500 μL DMEM in the presence of effectors at the same concentration. The reaction was terminated by aspirating the incubation medium, washing twice cells with 1 mL noradrenaline-free Locke's solution (composed of, in mM: NaCl 140, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KCl 4.7, KH_2PO_4 1.2, glucose 11, EDTA 0.01, ascorbic acid 0.546, HEPES 15, pH 7.5) and adding 0.5 mL of 0.4 M perchloric acid. Precipitated material was scraped off and centrifuged for 10 min at 10,000 *g* at 4°. Radioactivity from the clear supernatant was measured by liquid scintillation spectrometry [17].

Release studies. Prior to release experiments, cells were loaded with [7-^{3}H]noradrenaline as described above and washed six times (10 min each wash) with Locke's solution. Cells were then preincubated for 10 min at 37° in Locke's solution containing tuamine, heptaminol or analogues and stimulated with 10 μM nicotine in the presence of drugs. After 10 min stimulation, external medium was collected and the radioactivity was measured. Cells were precipitated with perchloric acid and scraped off the plates. [7-^{3}H]Noradrenaline release was calculated as the percentage of total radioactivity present in the cells at the beginning of experiment, assessed from the amount released plus the amount remaining in the cells [18].

Values are given as means ± SD. Linear regression equations were calculated by the least-squares method using linear regression programs.

RESULTS

Uptake of [7-^{3}H]*noradrenaline by chromaffin cells.*

In bovine chromaffin cells, two distinct uptake mechanisms are involved in the accumulation of exogenous noradrenaline. They differ in their apparent affinity for noradrenaline, ionic requirements and saturability [5, 6]. The high affinity process saturated at a noradrenaline concentration of 1 μM, is sensitive to cocaine, had an apparent K_m value of 0.55 ± 0.05 μM (N = 4) in our experimental conditions and exhibits an absolute Na^+ dependency. The second process has a low affinity for noradrenaline (K_m values are close to 90 μM, cf. Ref. 6), is not sensitive to cocaine, is not saturable and does not depend on Na^+ [5, 6, 19].

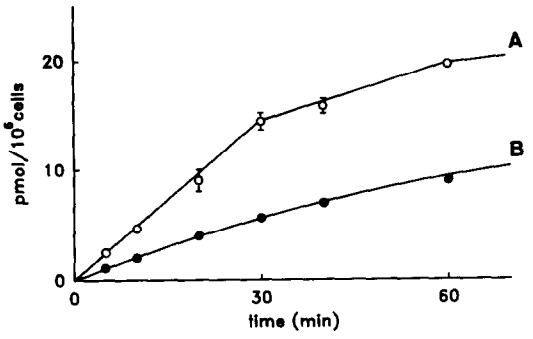

Fig. 1. Time course of noradrenaline uptake in the presence or absence of tuamine in bovine chromaffin cells in culture. Cells were incubated at 37° with 0.143 μM [7-^{3}H]noradrenaline in the absence (A: open circles), or presence (B: closed symbols) of 1 μM tuamine (1-methylhexylamine). Values are means (±SD) of three experiments in duplicate.

In the present study the concentration of external noradrenaline was fixed at 0.143 μM representing approximately 14% of the saturating concentration such that the noradrenaline uptake system was operating almost exclusively (>95%, see Ref. 20) via the Na^+-dependent, high-affinity, neuronal-like specific noradrenaline uptake process was measured. As shown in Fig. 1A, noradrenaline uptake rate linear during the first 30 min of incubation was calculated to be 0.4 pmol/min/10^6 cells. For all uptake experiments, the incubation time was fixed at 30 min.

Effects of tuamine, heptaminol, 1-methylamino-5-pentanol and 5-amino-2-methyl-2-hexanol on noradrenaline uptake in chromaffin cells

The uptake of noradrenaline was examined in the presence of tuamine, heptaminol and analogues. As shown in Fig. 1B, tuamine inhibited noradrenaline uptake. Uptake rate was linear during the first 30 min but decreased to 0.18 pmol/min/10^6 cells at a tuamine concentration of 1 μM. When noradrenaline uptake was determined in the presence of increasing tuamine concentrations (Fig. 2), noradrenaline uptake progressively decreased, inhibition reaching 90% at a tuamine concentration of 5 μM. IC_{50} (the concentration of tuamine producing 50% inhibition) was determined from Fig. 2 as 1 μM.

The inhibition provoked by tuamine was completely reversible since removal of tuamine from the external medium and washing of cells with tuamine-free medium abolished its inhibitory effect. To determine the inhibition pattern of tuamine, the inhibition was examined at different concentrations of noradrenaline ranging from 0.143 to 1 μM, the highest concentration being the saturating concentration of the high affinity, Na^+-dependent uptake process.

* Abbreviations: DMEM, Dulbecco's modified Eagle's medium supplemented with 0.546 mM ascorbic acid; heptaminol, 6-amino-2-methyl-2-heptanol; tuamine, 1-methylhexylamine; EDTA, ethylenediamine tetraacetate; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid.

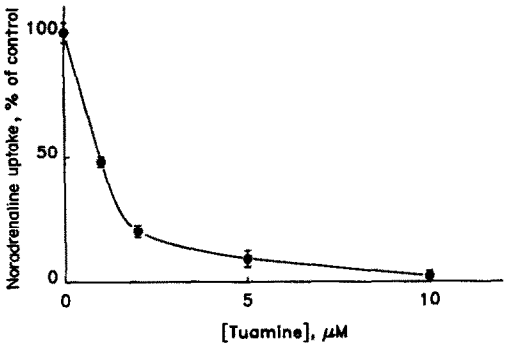

Fig. 2. Effect of tuamine on the noradrenaline uptake in chromaffin cells. Cells were incubated at 37° with 0.143 μM [7-^{3}H]noradrenaline for 30 min in the presence of tuamine at 1, 2, 5 and 10 μM. The uptake of [^{3}H]noradrenaline is expressed as per cent of the amount retained in cells in the absence of tuamine (control: 12 ± 0.5 pmol noradrenaline accumulated in 30 min per 10^6 cells = 100%). Values are means (±SD) of three experiments in duplicate.

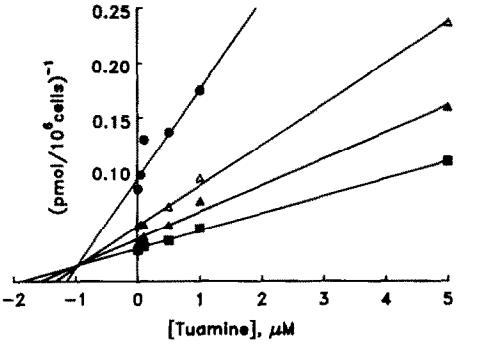

Fig. 3. Dixon plots of tuamine inhibition on noradrenaline uptake by chromaffin cells. Cells were incubated for 30 min at 37° in the presence of tuamine at the indicated concentration; [7-^{3}H]noradrenaline concentration was 0.143 μM (●—●), 0.34 μM (△—△), 0.57 μM (▲—▲) and 1 μM (■—■). Reciprocal uptake rate is expressed as (pmol noradrenaline taken per 10^6 cells)$^{-1}$. Each point represents the determination in duplicate on one chromaffin cell culture. This experiment was repeated twice with similar results on two different chromaffin cell cultures.

Table 1. Kinetic constants of high affinity noradrenaline uptake by and of noradrenaline release from bovine adrenal chromaffin cells in culture in the presence of 1-methylhexylamine (tuamine), 6-amino-2-methyl-2-heptanol (heptaminol), 1-methylamino-5-pentanol and 5-amino-2-methylhexanol. The values K_i and IC_{50} were the means (±SD) of three determinations, each performed on a different cell preparation

Compound	Uptake		Release
	IC_{50} (μM)	K_i (μM)	IC_{50} (μM)
1-Methylhexylamine (tuamine)	0.8 ± 0.1	1.1 ± 0.1	26 ± 2
6-Amino-2-methyl-2-heptanol (heptaminol)	50 ± 1	60 ± 2	650 ± 11
1-Methylamino-5-pentanol	160 ± 20	260 ± 28	—
5-Amino-2-methylhexanol	490 ± 90	480 ± 76	500 ± 10

Kinetic plots (Fig. 3) revealed that tuamine acts as a competitive inhibitor of noradrenaline uptake and had an inhibition constant $K_i = 1.1 \pm 0.1$ μM. Using a Lineweaver–Burk plot, the competitive nature of this inhibition was confirmed (data not shown).

Heptaminol, 1-methylamino-5-pentanol and 5-amino-2-methyl-2-hexanol were also found to affect noradrenaline uptake by chromaffin cells; similarly to tuamine, these analogues acted as competitive inhibitors (data not shown). However, as shown in Table 1, they were less potent than tuamine since their K_i values and IC_{50} values were *ca.* 60, 200 and 400 times higher, respectively. Thus, tuamine appears as a potent inhibitor of noradrenaline uptake in chromaffin cells. In this respect, its K_i value was similar to that of drugs such as cocaine and metaraminol acting on noradrenaline uptake in rat vas deferens ($K_i = 1.3$ μM) or chromaffin cells ($K_i = 1$ μM) and such as tricyclic antidepressants (chlorimipramine) in chromaffin cells [6, 20–24].

Effects of tuamine, heptaminol, 1-methylamino-5-pentanol and 5-amino-2-methyl-2-hexanol on catecholamine release from chromaffin cells

The effect of tuamine, heptaminol and their analogues on the nicotine-evoked release of catecholamine from chromaffin cells was also examined. Surprisingly, tuamine was found to exert a negative effect on catecholamine release evoked by 10 μM nicotine (Fig. 4). The IC_{50} was *ca.* 26 ± 2 μM. Heptaminol and 5-amino-2-methyl-2-hexanol were also able to inhibit catecholamine release, but they were less effective than tuamine, having IC_{50} values of 650 ± 10 μM and 500 ± 10 μM, respectively. In contrast, 1-methylamino-5-pentanol had no effect on nicotine-evoked catecholamine release from chromaffin cells (Table 1). A similar inhibition of release was also observed when carbamylcholine was used as the secretagogue. In contrast, when catecholamine release was evoked by a direct depolarization of cells with 59 mM K^+, neither tuamine (on the concentration range from 10 to 500 μM) nor heptaminol (from 10 μM to 0.1 mM) affected release.

To test the possibility that heptaminol might block the nicotine receptor, secretion was evoked by different nicotine concentrations. Increasing the nicotine concentration partially abolished the inhibiting effect of heptaminol on catecholamine release (Table 2). It was not possible to use nicotine concentration higher than 20 μM, as nicotine itself inhibits catecholamine release at these concentrations.

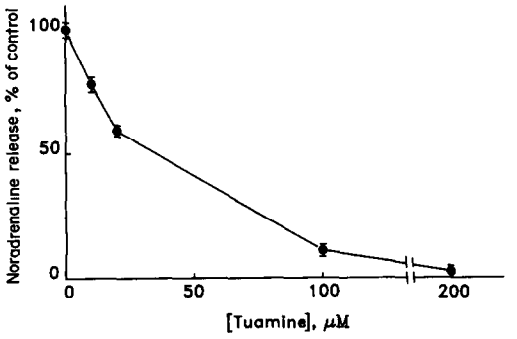

Fig. 4. Inhibition by tuamine of catecholamine release from cultured chromaffin cells. Cells were stimulated with 10 μM nicotine in the presence of tuamine; the tuamine concentration range was from 10 to 200 μM. [7-^{3}H]Noradrenaline release during stimulation periods was calculated as the percentage of the total radioactivity present in the cells prior to stimulation. Results are expressed relative to the response obtained when stimulation was performed in the absence of tuamine (21.5 ± 0.5% of total [^{3}H]noradrenaline cell content) taken as 100%. Each point represents the mean value (±SD) of three experiments, in duplicate, each performed on a different cell preparation.

Table 2. Partial reversion of heptaminol-induced inhibition of catecholamine release by nicotine

	Heptaminol (M)		
Nicotine (μM)	0	10^{-4}	5×10^{-4}
5	100	75	77
10	100	46	63
20	100	49	45

Chromaffin cells were stimulated with nicotine at the indicated concentration in the presence of heptaminol. Secretion in the absence of heptaminol was 16.5, 18.75 and 17.0% of total stored catecholamines at 5, 10 and 20 μM nicotine, respectively. Results are expressed relative to the response obtained when stimulation was performed in the absence of heptaminol (control = 100% for each nicotine concentration). This experiment was repeated once with similar results on a different cell preparation. Each point has been performed in duplicate.

DISCUSSION

At the low exogenous noradrenaline concentration used in the present study (0.143 μM), noradrenaline uptake in cultured bovine chromaffin cells has been shown to be 95% sodium-dependent [20]. This implies that tuamine, heptaminol and their derivatives inhibited the specific noradrenaline uptake system described as the high-affinity, cocaine-sensitive and saturating, neuronal-like uptake process [5, 6]. The possible effects of these compounds on the cocaine-insensitive, sodium-independent, unspecific uptake process, which probably occurs by a simple diffusion [20], were not examined in the present study.

Grobecker and Grobecker [12] have reported that heptaminol increases spontaneous release of catecholamines from isolated chromaffin granules and also reduces uptake of adrenaline by isolated chromaffin granules. However, the rather high concentration of heptaminol ($> 10^3$ M) necessary to induce the spontaneous release of catecholamines indicates that the pharmacological action of heptaminol is unlikely to involve displacement of granule amines. Our findings indicate that part of the pharmacological effect of heptaminol may result from an inhibition of catecholamine uptake, which thereby potentiates the peripheral action of catecholamines on target tissues.

Surprisingly, tuamine, heptaminol and the related compound 5-amino-2-methyl-2-hexanol were found to inhibit catecholamine release from cultured chromaffin cells. Comparison of the molecular structures of tuamine, heptaminol, 1-methylamino-5-pentanol and 5-amino-2-methyl-2-hexanol suggests that the amino group is required for the inhibition of catecholamine uptake and that substitution on the alkyl chain with methyl and hydroxyl groups reduces the inhibition of both catecholamine uptake and release. However, with the exception of tuamine, the inhibition constants for catecholamine release were rather high, which casts some doubt on the pharmacological importance of these observations. Because of the high concentrations required to inhibit release, non-specific cell membrane alterations can not be excluded; in this respect, heptaminol at 1–10 mM has been reported to block the contractile response of the mouse diaphragm [25].

The lack of effect of heptaminol and tuamine on high K^+-evoked catecholamine release from chromaffin cells indicates that these compounds may act at a stage prior to calcium entry [26, 27]. The partial reversal of heptaminol-induced inhibition with increasing nicotine concentrations, suggests that heptaminol might be a weak blocker of the nicotine receptor on chromaffin-cells. Further studies involving electrophysiological measurements of nicotine receptor-associated currents are required to confirm this interpretation.

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