Influence of different anions on tyramine and amphetamine uptake by cow adrenal medulla chromaffin vesicles

Catecholamines are accumulated in peripheral sympathetic nerve terminals by active uptake at the cell membrane and by uptake and binding in the intraneuronal storage vesicles. Both processes are energy dependent, exhibit saturable kinetics and can be inhibited by various drugs (Iversen, 1973). The major storage site for catecholamines in both sympathetic nerve terminals and in adrenal medulla is these storage vesicles.

Isolated chromaffin vesicles from the cow adrenal medulla have been used to study catecholamine release induced by tyramine and amphetamine (Wagner, Koerker & Schneider, 1973) the catecholamine releasing ability of which is markedly affected by the type of anion predominating in the incubation medium. Both amines release catecholamines in media containing carbonate, bicarbonate or phosphate as the primary anion, but not in sulphate or chloride-containing media. The anions may produce their effects by differentially altering the uptake of the releasing agent into the chromaffin vesicle, the stability of the catecholamine storage complex within the vesicle or diffusion of the catecholamines out of the storage vesicle. We report on the first of these possibilities.

The large vesicle fraction (LVF) was prepared from fresh cow adrenal medullae (30 g) homogenized with a loose-fitting Potter-Elvehjem homogenizer in a volume of incubation medium equal to four times the wet weight of tissue. Homogenates were prepared in the same isosmotic medium in which the corresponding LVF was to be incubated. The incubation media used were (M): NaCl 0·15; Na_2SO_4 0·10; NaH_2PO_4 0·10; $NaHCO_3$ 0·15; Na_2CO_3 0·10 and Tyrode solution (Schneider, Smith & Winkler, 1967). All media were gassed with 5% carbon dioxide in oxygen and then adjusted to pH 7·0 with HCl or NaOH. The homogenate was centrifuged at 480 *g* for 10 min. The supernatant was centrifuged at 12 000 *g* for 20 min, and the sediment resuspended in incubation medium and centrifuged again at 12 000 *g* for 20 min. The chromaffin vesicle fraction was obtained by suspending the resulting pellet in a volume of incubation medium equal to ten times its wet weight.

Uptake of [^{3}H]tyramine hydrochloride and [^{3}H]amphetamine sulphate (specific activity: 7·3 and 8·3 Ci mmol^{-1}, respectively; New England Nuclear) by the LVF was measured by adding 1 ml of the LVF to 1 ml of appropriate incubation mixture containing the radioactive amine. After incubation for 12 min at either 0° or 30° in 5% carbon dioxide in oxygen, the suspension was centrifuged for 15 min at 27 000 *g* at 4° and an aliquot of the supernatant counted for radio activity by liquid scintillation spectrophotometry in a mixture of 2 parts of toluene scintillation fluid (2,5-diphenyl-oxazole 5, and 1,4 bis[2-(4-methyl-5 phenyl oxazolyl)]benzene 0·3 g litre^{-1}) and 1 part Triton X-100. The pellet was washed 3 times with the incubation medium (not containing radioactive amine) and extracted with 0·4N perchloric acid; the extract was counted for radioactivity in the same scintillation fluid. Counts per min were corrected for counting efficiency and quenching and expressed as disintegrations per min. Uptake was expressed as nmol of amine g^{-1} LVF or as the ratio nmol g^{-1} LVF: nmol ml^{-1} medium. The metabolism of either tyramine or amphetamine was not assessed.

Density gradient centrifugation of the LVF preparation was carried out over gradients of 1·5 ml 2·5M sucrose, 8·5 ml of either 1·7M or 1·6M sucrose and 4 ml of 1·3M sucrose according to Schneider (1972). Fractions were assayed for catecholamines, protein, β-glucuronidase activity and fumarase activity (Schneider, 1972).

As initially uptake of [^{3}H]tyramine ($1{\cdot}4 \times 10^{-4}$M) or [^{3}H]amphetamine (10^{-4}M) at 30° in Tyrode solution was maximal at 3 min and remained constant up to 24 min with the tissue: medium ratios of 2–3 for tyramine but only 0·15–0·25 for amphetamine,

incubation periods of 12 min were used. Tritium, arbitrarily expressed as nmol [^{3}H]tyramine g^{-1} LVF, was accumulated in the LVF in a concentration-dependent manner over the range $6{\cdot}5 \times 10^{-9}$ to 5×10^{-4}M tyramine ($r = 1{\cdot}0$, $n = 6$) although the tissue : medium ratio was constant (between 1·9 and 2·6). Tyramine uptake in Tyrode solution was 6 times greater throughout this concentration range at 30° than at 0°. Likewise, a positive correlation ($r = 1{\cdot}0$, $n = 4$) existed between the uptake of amphetamine in Tyrode solution and the concentration of amphetamine in the medium, although the tissue : medium ratios were much less than for tyramine, varying between 0·2 at $6{\cdot}5 \times 10^{-9}$ and 0·3 at $6{\cdot}5 \times 10^{-4}$M. Therefore, there was no net uptake of amphetamine by the vesicles. There was no difference between uptake of amphetamine at 0° and at 30°. Previous investigators (see Iversen, 1973) have demonstrated that *N*-ethylmaleimide, a nonspecific inhibitor of sulfhydryl group-containing enzymes, inhibits the vesicle uptake process and that the addition of ATP and Mg^{2+} stimulates catecholamine uptake. We found *N*-ethylmaleimide (10^{-4}M) did not significantly alter the uptake of either tyramine or amphetamine into the LVF incubated in Tyrode solution. Similarly, the addition of Mg^{2+} and ATP (0·5 and 5 mM, respectively) to the incubation failed to alter uptake of the amines. Since the LVF is not a pure fraction and contains mitochondria, in which monoamine oxidase is located, the ability of the monoamine oxidase inhibitor pargyline (10^{-4}M) to alter uptake was examined. It caused an increase (60%) in the uptake of [^{3}H]tyramine but had no effect on amphetamine uptake.

The uptake of tyramine by the LVF incubated in Na_2CO_3 increased in a dose-dependent manner (Fig. 1a) and was greater at 30° than at 0°. However, the tissue: medium ratio was similar at all doses tested; tyramine was not concentrated at 0°, but was markedly concentrated at 30° (Fig. 1b). However, the net uptake of amphet-

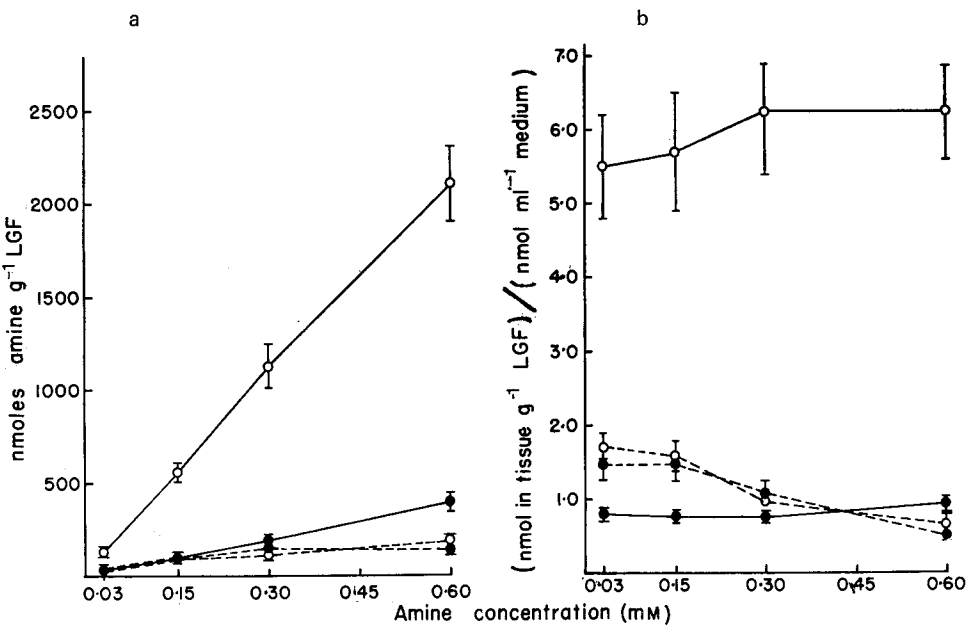

FIG. 1. Uptake (a) and tissue: medium ratio (b) of [^{3}H]tyramine (●——●, 0°; ○——○ 30°) and [^{3}H]amphetamine (●- - -●, 9, 0°; ○- - -○, 9, 30°) in the LVF of the cow adrenal medulla incubated 12 min in Na_2CO_3 (0·1M, pH 7·0) at 30° and 0°. Ordinates show uptake expressed as nmol of amine g^{-1} LVF (a) and the ratio nmol g^{-1} LVF: nmol ml^{-1} medium (b). Abscissae show molar concentrations of the amines. Mean values (± s.e.) for uptake are shown ($n = 3$).

Table 1. *Uptake of tyramine and amphetamine at 30° and 0° in different media.*

Medium	Amine concentration (M)	Tyramine 30°	Tyramine 0°	Amphetamine 30°	Amphetamine 0°
$NaHCO_3$	3×10^{-5}	57·1 ± 10·1 (2·6 ± 0·5)	20·1 ± 3·0 (0·8 ± 0·1)	13·5 ± 0·5 (1·0 ± 0·1)	12·2 ± 1·9 (1·1 ± 0·2)
	6×10^{-4}	437·6 ± 116·0 (1·5 ± 0·4)	402·8 ± 134·8 (1·2 ± 0·3)	77·3 ± 1·9 (0·2 ± 0·0)	59·6 ± 4·4 (0·2 ± 0·0)
NaH_2PO_4	3×10^{-5}	33·8 ± 3·0 (1·3 ± 0·1)	9·7 ± 4·0 (0·3 ± 0·1)	4·1 ± 0·4 (0·2 ± 0·0)	2·3 ± 0·3 (0·1 ± 0·0)
	6×10^{-4}	479·8 ± 72·0 (1·2 ± 0·1)	89·5 ± 7·0 (0·2 ± 0·0)	50·2 ± 4·7 (0·1 ± 0·0)	30·3 ± 3·5 (0·1 ± 0·0)
NaCl	3×10^{-5}	37·5 ± 5·0 (1·6 ± 0·2)	3·2 ± 0·0 (0·1 ± 0·0)	4·1 ± 0·2 (0·2 ± 0·0)	1·8 ± 0·2 (0·9 ± 0·0)
	6×10^{-4}	455·7 ± 0·1 (1·3 ± 0·1)	91·2 ± 13·0 (0·2 ± 0·0)	44·5 ± 2·0 (0·1 ± 0·0)	23·8 ± 2·7 (0·6 ± 0·0)
Na_2SO_4	3×10^{-5}	45·1 ± 12·0 (1·9 ± 0·6)	5·2 ± 3·0 (0·2 ± 0·1)	3·8 ± 1·0 (0·2 ± 0·0)	1·6 ± 0·3 (0·1 ± 0·0)
	6×10^{-4}	410·0 ± 158·0 (1·2 ± 0·6)	89·6 ± 42·0 (0·2 ± 0·1)	42·7 ± 8·1 (0·1 ± 0·0)	26·3 ± 5·3 (0·1 ± 0·0)

Mean values (± s.e., n = 3) for uptake of two concentrations of tyramine or amphetamine are expressed as nmol g^{-1} LVF. The ratio, nmol g^{-1} LVF: nmol ml^{-1} medium, is shown in parentheses.

amine in Na_2CO_3 did not increase with increasing concentration (Fig. 1a) and was much less than for tyramine. At low doses, there was a slight concentration of amphetamine in the tissues, an effect not seen at higher doses (Fig. 1b). There was no difference in the uptake at 0° and at 30°.

The uptake of tyramine was also greater than the uptake of amphetamine in the other media examined and, with the exception of $NaHCO_3$, was greater at 30° than at 0° (Table 1). Amphetamine was not concentrated by the vesicles in any of the other media and temperature did not affect uptake (Table 1).

Density gradient centrifugation of the LVF after incubation with [H^3]tyramine ($6{\cdot}2 \times 10^{-8}$M) for 12 min at 30° in Tyrode solution showed that the distribution of [^{3}H] was similar to the distribution of catecholamines and different from the distribution of β-glucuronidase and fumarase, indicating that the [^{3}H]tyramine was associated primarily with chromaffin vesicles.

The following conclusions can be formed:

1. Uptake and retention of tyramine by isolated chromaffin vesicles of the cow adrenal medulla is much greater than uptake and retention of amphetamine: this uptake process is different from that for adrenaline, since its uptake by chromaffin vesicles is stimulated by Mg^{2+} and ATP. Apparent binding of [^{3}H]tyramine is probably not due to binding of a metabolite since inhibition of monoamine oxidase, which is capable of oxidative deamination of tyramine, did not decrease the uptake.

2. Uptake of tyramine is reduced at low temperature, whereas uptake of amphetamine is generally unaffected by temperature. This is consistent with uptake of amphetamine by a diffusion process which does not require energy.

3. Of the media studied, uptake of both amines was greatest in Na_2CO_3.

The effects of the anions on uptake of the amines differ from the effects on the release of catecholamines from chromaffin vesicles by these two agents in three important respects. First, although amphetamine is a more potent releasing agent than tyramine in all the media it is taken up much less, suggesting that amphetamine may be acting on the chromaffin vesicle in a different manner from tyramine. Second, neither amine releases catecholamines in Na_2SO_4 or in NaCl, although both are taken up to the same extent in these media as they are in the other media. Third, release of catecholamines by either amine was similar for incubation in $NaHCO_3$ and Na_2CO_3, but uptake was 4-fold less in $NaHCO_3$ than in Na_2CO_3. It appears that the various anions affect release of catecholamines by mechanisms other than by altering the uptake of the releasing agent.

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The role of solvent interaction in quantum pharmacological studies

Molecular orbital (MO) studies of pharmacological agents are becoming an increasingly important area of research (cf. Kier, 1971, 1972). However, the physical model from which the quantum mechanical methods are derived probably resembles the molecule most nearly as it exists in the vapour phase. Consequently, the question arises as to whether the results of MO studies are valid for the molecule in solution, the physical state of greatest interest to the pharmacologist. The general agreement between calculated configurations and those deduced from crystallographic and/or nmr data, when available, has been used as an argument that the calculated results are applicable to the more interactive states. In this note, however, the results of a different approach to this question are reported. The basic Hartree-Fock (H-F) self-consistent field (SCF) MO formalism described by Roothaan (1951) has been applied to two classical physical models of a molecule in solution. A conformational study of noradrenaline (protonated) has then been made, incorporating each of the two