The role of co-transported sodium in the effect of indirectly acting sympathomimetic amines*

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Summary. The adrenergic nerve endings of vasa deferentia of either untreated or reserpinated (R) and/or pargyline (P) pretreated rats were loaded with $^{3}$H-noradrenaline; COMT was inhibited by U-0521 (U). After 100 min of wash-out with Ca$^{2+}$-free solution, the efflux of tritium (and of $^{3}$H-noradrenaline) from the tissue was largely of neuronal origin and remained constant with time (when expressed as fractional rate of loss; FRL). After 110 min of wash-out the effect of inhibition of the Na$^{+}$,K$^{+}$-ATPase (by low K$^{+}$ or ouabain) on basal and on sympathomimetic amine-induced efflux of tritium (or $^{3}$H-noradrenaline, under the condition U) was studied in paired experiments.

Inhibition of the Na$^{+}$,K$^{+}$-ATPase caused a time-dependent increase in the efflux of tritium (or $^{3}$H-noradrenaline) which was inhibited by desipramine.

Inhibition of the Na$^{+}$,K$^{+}$-ATPase also caused a time-dependent reduction of the initial rate of neuronal uptake of $^{3}$H-noradrenaline.

The effectiveness of the sympathomimetic amines tyramine and amphetamine in inducing "release" (i.e., outward-transport) of noradrenaline depended on the experimental condition: it was most pronounced under the condition RPU followed by the condition PU and lowest under the condition U (i.e., in tissue of untreated rats). Inhibition of the Na$^{+}$,K$^{+}$-ATPase caused an early and transient enhancement of the "release" of noradrenaline induced by tyramine or amphetamine. This enhancement was seen already within the first min after inhibition of the ATPase, i.e., before a pronounced inhibition of uptake (of noradrenaline) and before a pronounced increase of the basal efflux was observed. It also depended on the experimental condition: RPU > PU > U; i.e., it was the more pronounced, the higher the free axoplasmic concentration of noradrenaline.

In tissues of untreated rats, tyramine increased the rate of efflux of DOPEG, whereas amphetamine decreased it.

Conclusions. 1) Both, tyramine and amphetamine are transported by the Na$^{+}$-dependent neuronal transport system; 2) the co-transported Na$^{+}$ causes a local increase in the Na$^{+}$ concentration at the inside of the neuronal plasma membrane and thereby contributes to the outward-transport of axoplasmic noradrenaline induced by indirectly acting sympathomimetic amines; however, this contribution is only of importance when the axoplasmic concentration of noradrenaline is high (RPU, PU).

Key words: Sympathomimetic amines — Efflux of noradrenaline — Neuronal uptake — Amphetamine — Tyramine — Na$^{+}$—K$^{+}$-ATPase inhibition

Introduction

Tyramine is transported by the Na$^{+}$-dependent neuronal amine transport system, and it is commonly accepted that the outward transport of noradrenaline induced by this amine is mediated by "facilitated exchange diffusion"; i.e., the inward transport of this amine increases the proportion of carrier molecules on inside of the neuronal plasma membrane and thus induces an outward transport of axoplasmic noradrenaline (for review see Trendelenburg 1978). Due to a lack of evidence for carrier-mediated uptake of amphetamine (see e.g., Thoenen et al. 1968), the efflux of noradrenaline by amphetamine has hitherto often been explained by inhibition of reuptake of noradrenaline and/or inhibition of monoamine oxidase (MAO). Recently it has been shown, that amphetamine is transported by the neuronal amine carrier (Bönisch 1984). Hence, it was of interest to reinvestigate the efflux of noradrenaline induced by amphetamine in comparison to that induced by tyramine. It was of special interest to examine, by inhibition of the Na$^{+}$,K$^{+}$-ATPase, whether the co-transported Na$^{+}$ contributes to the action of sympathomimetic amines.

According to Sammet and Graefe (1979) the inward transport of any substrate of the neuronal amine transport system goes hand in hand with a co-transport of sodium. This may lead to an increase of the sodium concentration at the inside of the axonal membrane. Sammet and Graefe (1979) demonstrated that any increase in the inside sodium concentration should a) increase the availability of the carrier on the inside of the axonal membrane, and b) decrease the $K_m$ for the outward transport of noradrenaline. Thus, the co-transport of sodium (together with the inward transport of indirectly acting sympathomimetic amines) might be able to increase the noradrenaline-releasing effects of these amines. This hypothesis was tested by the determination of the noradrenaline-releasing effect of tyramine and amphetamine when the Na$^{+}$,K$^{+}$-ATPase was inhibited. Such inhibition should reduce the noradrenaline-releasing effect of indirectly acting sympathomimetic amines, when the inside sodium concentration has risen to such a height that the sodium gradient is clearly diminished. However, this is a time-consuming process. If the co-transported sodium is

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involved in the releasing effect of these amines, the eventual inhibition of the effect of indirectly acting sympathomimetic amines should be preceded by a transient facilitation of release (as a consequence of the lack of outward pumping of sodium by the Na⁺,K⁺-ATPase).

Methods
The experiments were carried out under three different experimental conditions: RPU, PU, and U (for abbreviations, see below). Vasa deferentia were taken from untreated rats or from rats pretreated with either pargyline (P; to inhibit monoamine oxidase; 100 mg/kg i.p. 3 h prior to experiment) or pargyline and reserpine (R; to deplete the stores of endogenous noradrenaline; 5 mg/kg s.c. 20 h and 2.5 mg/kg 3 h prior to experiment). The middle portion of the tunica muscularis of the vas deferens was incubated with 2 ml of incubation medium at 37°C (for details see Keller and Graefe 1979).

The medium was Ca²⁺-free and had the following composition (in mmol/l): NaCl 118.0, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25.0, D-glucose 5.0, Na₂EDTA 0.04, ascorbic acid 0.57, and 3,4-dihydroxy-2-methyl propiophenone (U-0521; U; to inhibit catechol-O-methyl transferase) 0.1. The medium was bubbled with 95% O₂ and 5% CO₂; its final pH was 7.4. To inhibit the Na⁺,K⁺-ATPase, either ouabain was added to the medium or the concentration of K⁺ was reduced to a low concentration after 110 min of wash-out.

Results

A. Effect of inhibition of Na⁺,K⁺-ATPase on neuronal uptake of ³H-noradrenaline

First, the effect of inhibition of Na⁺,K⁺-ATPase on neuronal uptake of ³H-noradrenaline was studied. Pairs of vasa deferentia from rats pretreated with reserpine (R, to inhibit vesicular uptake) and pargyline (P, to inhibit MAO) were exposed for 1 min to 0.5 μmol/l ³H-noradrenaline, one in the absence, the other in the presence of cocaine (30 μmol/l). The cocaine-sensitive uptake of ³H-noradrenaline was regarded as carrier-mediated neuronal uptake (Keller and Graefe 1979).

Results are expressed as arithmetic means (± SEM), or (for factors) as geometric means (with 95% confidence limits in parenthesis). The significance of differences was calculated by means of the t-test (Snedecor and Cochran 1980).

Agents used in this study: pargyline hydrochloride, reserpine, (+)-amphetamine sulphate (Sigma, St. Louis, MO, USA); cocaine hydrochloride, ouabain (Merck, Darmstadt, FRG); desipramine hydrochloride (Ciba-Geigy, Basel, Switzerland); tyramine hydrochloride (Serva, Heidelberg, FRG); 3,4-dihydroxy-2-methyl-propiophenone (U-0521; Upjohn, Kalamazoo, MI, USA); (-)-noradrenaline-7-3H(N) (166.5 GBq/mmol; lot No 1271-019; NEN Chemicals, Dreieich, FRG).

In all experiments the change of the basal efflux of tritium or ³H-noradrenaline (induced by agents or procedures after 110 min of wash-out) was expressed as "increase of the FRL" over basal FRL. The basal FRL was determined as the FRL of untreated, untreated vasa deferentia not exposed to ³H-noradrenaline. The change of the FRL was expressed as percent of the amount of tritium present in the tissue at the end of the experiment (Graefe et al. 1973, 1977). Since COMT was largely inhibited by U-0521, only three fractions were separated: noradrenaline (NA), dihydroxyphenyl ethyleneglycol (DOPEG) and dihydroxymandelic acid (DOMA). Irrespective of whether the rats were untreated or pretreated with reserpine and/or pargyline, more than 90% of the tritium present in the tissue at the end of the experiment represented unchanged noradrenaline.

In one series of experiments initial rates of neuronal uptake of ³H-noradrenaline were determined. For this purpose pairs of vasa deferentia (of reserpine and pargyline pretreated rats) were incubated at 37°C for 1 min with 0.5 μmol/l ³H-noradrenaline, one in the absence, the other in the presence of cocaine (30 μmol/l). The cocaine-sensitive uptake of ³H-noradrenaline was regarded as carrier-mediated neuronal uptake (Keller and Graefe 1979).

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