Influence of Phenylephrine and Orciprenaline on the Release of Noradrenaline

Several α-receptor-stimulant drugs, including noradrenaline, diminish the release of noradrenaline from sympathetic neurones in response to nerve impulses[1–6]. They probably act on α-receptors which may be localized in the nerve terminals and somehow influence the release process. Liberated extracellular noradrenaline will thus inhibit the secretion of noradrenaline during subsequent impulses. In the present paper the effects of 2 further sympathomimetic agents on the secretory response to sympathetic nerve stimulation are described: phenylephrine, which acts predominantly on α-receptors, and orciprenaline, which acts predominantly on β-receptors. Moreover, the interaction of either drug with high concentrations of the α-adrenolytic agent, phenoxybenzamine, and the β-adrenolytic agent, propranolol, has been tested.

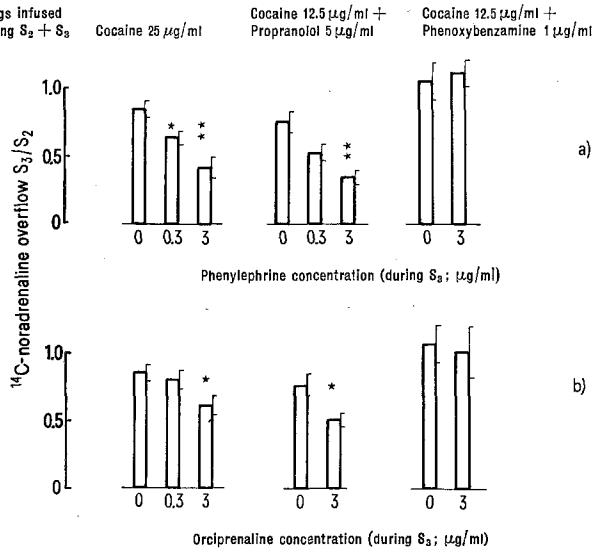
Methods. The experiments were done in isolated perfused rabbit hearts. The cardiac noradrenaline stores were labelled by 15 min perfusion with 50 ng/ml of (±)-β-^{14}C-noradrenaline, s.a. 44 Ci/mole. Starting 20 min later, the cardiac sympathetic nerves[7] were stimulated 3 times for 1 min with intervals of 15 min (3 msec, 8 mA). In general, the stimulation frequency was 5 Hz; however, it was reduced to 2.5 Hz in phenoxybenzamine experiments, because phenoxybenzamine greatly elevates the stimulation-evoked release of noradrenaline, and because a large release of noradrenaline per se decreases the inhibitory effect of sympathomimetic drugs[4]. In the venous effluent, ^{14}C-noradrenaline and total radioactivity were determined. The stimulation-induced overflow of radioactive material was calculated as the difference between the overflow during and plus the first 3 min after stimulation, and the overflow during the 4 min before stimulation. Only the outflow of ^{14}C-noradrenaline is reported here; however, an evaluation of the outflow of total radioactivity gave analogous results. For details of methods, see[5].

Results. In preliminary experiments it was found that phenylephrine, even at the lowest concentration tested (30 ng/ml), accelerated the spontaneous efflux of ^{14}C-noradrenaline (i.e., in the absence of nerve impulses, cf.[8]). In all subsequent experiments, cocaine was infused 16 min prior to and during the infusion of phenylephrine at a concentration sufficient to prevent this 'indirect sympathomimetic' release of noradrenaline. Orciprenaline in concentrations of up to 3 μg/ml did not augment the spontaneous outflow of ^{14}C-noradrenaline. Nevertheless, cocaine was also infused in orciprenaline experiments in order to obtain analogous conditions.

The influence of phenylephrine and orciprenaline on the response to stimulation is illustrated in the Figure. In the absence of adrenolytic drugs (left-hand group of columns), phenylephrine depressed the stimulation-induced overflow of ^{14}C-noradrenaline already at a concentration of 0.3 μg/ml. The inhibitory effect was not prevented by pre-infusion of propranolol (middle group of columns), but was blocked by pre-infusion of phenoxybenzamine (right-hand group of columns). In contrast to phenylephrine, orciprenaline diminished the stimulation-induced overflow only at the high concentration of 3 μg/ml (left-hand group of columns). Again, the effect was blocked by phenoxybenzamine, but not by propranolol.

Discussion. At appropriate concentrations both phenylephrine and orciprenaline depress the overflow of ^{14}C-noradrenaline (and total radioactivity, not shown) in response to sympathetic nerve stimulation. It is unlikely that the drugs increase the retention of liberated noradrenaline within the heart; therefore, it can be assumed that they depress the overflow of ^{14}C-noradrenaline by decreasing its release from the nerve terminals.

This decrease is obtained at a low concentration of phenylephrine, but only at a high concentration of orciprenaline; moreover, it can be blocked by an α-adrenolytic, but not by a β-adrenolytic drug. Thus, the inhibition seems to be mediated by structures similar to the α-receptors of effector cells. The relatively weak effect of orciprenaline may be due to some inherent α-receptor-stimulant activity. The results are in accord with the idea that the stimulation evoked liberation of the adrenergic transmitter can be modulated via α-receptors, and is feed-back inhibited by liberated noradrenaline.



Influence of phenylephrine and orciprenaline on the stimulation-evoked overflow of ^{14}C-noradrenaline from isolated rabbit hearts pre-perfused with (±)-^{14}C-noradrenaline. The sympathetic cardiac nerves were stimulated 3 times for 1 min with intervals of 15 min (S_1–S_3). Cocaine (left-hand group of columns), or cocaine + propranolol (middle group of columns), or cocaine + phenoxybenzamine (right-hand group of columns) were infused from 8 min before S_2, and phenylephrine (a) or orciprenaline (b) were infused from 8 min before S_3 until the end of the experiment. Results are expressed as the ratio between the overflow of ^{14}C-noradrenaline induced by S_3 (in the presence of the sympathomimetic drugs) and that induced by S_2 (before the infusion of the sympathomimetic drugs). Each column is the mean ± S.E.M. of 4 experiments. Significant differences from corresponding controls (sympathomimetic drug concentration = 0): * $P < 0.05$; ** $P < 0.02$.

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Zusammenfassung. Die Wirkung von Phenylephrin und Orciprenalin auf die Freisetzung von Noradrenalin wurde an isolierten, perfundierten Kaninchenherzen mit ^{14}C-markierten Noradrenalin-Vorräten untersucht. Beide Stoffe verminderten die Abgabe von ^{14}C-Noradrenalin bei Sympathicusreizung. Diese Hemmwirkung wirde durch Vorinfusion von Phenoxybenzamin, nicht aber durch Vorinfusion von Propranolol verhindert.

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On the Origin of Small Adrenergic Storage Vesicles: Evidence for Local Formation in Nerve Endings after Chronic Reserpine Treatment

The axonal enlargements (varicosities, nerve endings) of peripheral autonomic nerves contain large amounts of vesicles supposed to represent the intraneuronal storage sites for the transmitter substance[1]. In this paper some preliminary findings are presented, demonstrating that chronic treatment with reserpine, a drug known to deplete monoamine stores in the peripheral and central nervous system[2], causes certain fine structural changes in peripheral adrenergic nerve endings in the dilator muscle of the rat iris. These changes are discussed in relation to the formation of storage vesicles.

Material and methods. Ten male albino rats (Sprague-Dawley, b. wt. 200 g) were used, 8 of which received 1 daily dose of reserpine (Serpasil®, 1 mg/kg i.p.) for 6 days. 4 rats were sacrificed 24 h and 4 rats 72 h after the last injection. 2 untreated rats served as controls. The irides were fixed in ice cold 3% potassium permanganate ($KMnO_4$)[3] and processed for electron microscopy as previously described[4].

Results and discussion. Two types of axons were seen in untreated rats, adrenergic and presumably cholinergic, characterized respectively by their content of granular (vesicles with an electron dense core) (DCV) and agranular vesicles (Figure 1) with a spherical or slightly flattened shape and a diameter mainly of about 500 Å. In addition, both types of varicosities contain mitochondria and a few tubular or irregular membrane structures probably belonging to the axonic smooth endoplasmic reticulum (ASER) (Figure 1).

Both 24 and 72 h after the last injection of reserpine, several varicosities are seen containing many 'elongated vesicles'[5], tubular and/or slightly hour-glass formed membrane structures (thickness about 150–300 Å) (Figures 2 and 3) from which vesicles occasionally seem to bud off. Sometimes an electron dense precipitate is found within these structures (Figure 3). Such varicosities contain in addition 'normal' spherical vesicles, some of which may have an electron dense core (Figure 2). Other varicosities have almost exclusively spherical vesicles many of which may contain an electron dense core, especially 72 h after the last reserpine injection. The presence of DCV strongly suggests that these nerve endings belong to adrenergic neurons (for ref. see[6]). In varicosities with many tubular structures and 'elongated vesicles' the proportion of spherical DCV is low. Since the number and proportion of DCV probably parallel the recovery of NA levels after the reserpine treatment, it may be assumed that such nerve endings are in an early phase of recovery. Further varicosities contain vesicles only of the agranular type and are indistinguishable from those present in untreated rats and presumed to belong to cholinergic neurons. No obvious increase in the number of tubular structures and 'elongated vesicles' was found in this type of varicosity, indicating that reserpine causes such changes only in adrenergic neurons.

Although several explanations may be advanced we would like to discuss, hypothetically, the present results in relation to the formation of storage vesicles. Almost all components of the neuron have been proposed as the origin of the synaptic vesicles: Mitochondria[7], microtubules[8], nerve cell membrane and complex vesicles[9–13], large granular vesicles[14], the Golgi apparatus[15] and finally the ASER[15–19]. For an extensive discussion and further ref. see[18].

Flat vesicles have previously been described in normal[20] and reserpine treated[21] animals. In the present study an increase in the number of 'elongated vesicles' and tubular structures probably belonging to the ASER was observed specifically in adrenergic nerve terminals 24 and 72 h after chronic treatment with reserpine, a drug which is known to cause a long-lasting depletion of amine levels. Since this depletion probably is due to an irreversible destruction of the Mg^{++}-ATP dependent storage mechanism, the

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