In vivo microdialysis of noradrenaline overflow: effects of α -adrenoceptor agonists and antagonists measured by cumulative concentration-response curves

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1 The purpose of the present study was to compare the effects of several α -adrenoceptor agonists and antagonists on cerebral cortical overflow of endogenous noradrenaline (NA) in freely moving rats. One or two days after the implantation of transcerebral dialysis tubes in the frontoparietal cortex, extracellular NA levels were monitored on-line with high performance liquid chromatography and electrochemical detection. The drugs were applied locally via the dialysis membrane, and effects on NA overflow were determined in cumulative concentration-response curves.

2 The average basal cortical NA overflow of all experiments was 0.25 pg min^{-1} . The α_2 -adrenoceptor agonists caused a concentration-dependent decrease in NA levels. UK-14,304 was the most potent and B-HT 933 the least potent agonist. The maximal decrease in NA overflow was to 10–15% of control levels after UK-14,304 or moxonidine, to 30% after clonidine and to 50% after B-HT 933 administration. Continuous activation of the presynaptic α_2 -adrenoceptor with 10^{-6} M UK-14,304 caused a decrease in NA levels to 40–50% of basal levels. This decrease was reached within 1 h and remained stable for the entire 3 h measurement period. The α_1 -adrenoceptor agonists, phenylephrine and methoxamine, induced an increase in NA levels to 225% and 300%, respectively, at a concentration of 10^{-3} M .

3 Local application of α_2 -adrenoceptor antagonists caused an increase in NA levels, with idazoxan being more potent than piperoxan. Yohimbine did not cause any significant change.

4 All drugs used in these *in vivo* experiments had *in vitro* recoveries across the dialysis membrane between 10 and 20%.

5 We conclude that microdialysis with local drug application is suitable for the comparison of the pharmacological effects of drugs with affinity for α -adrenoceptors on cortical NA overflow *in vivo*, provided that the passage across the membrane is equal for the different drugs.

Keywords: Microdialysis; noradrenaline overflow; α_2 -autoreceptor; clonidine; B-HT 933; moxonidine; UK-14,304; idazoxan; piperoxan; yohimbine

Introduction

Several neurotransmitters can modulate their own release via a negative feedback mechanism mediated by presynaptic autoreceptors (Starke *et al.*, 1989). Receptor-mediated modulation of peripheral noradrenaline (NA) release in various *in vitro* and *in vivo* preparations has been described extensively (reviews by Gillespie, 1980; Langer, 1981; Starke, 1981; 1987). Evidence that NA overflow is modulated by presynaptic α_2 -autoreceptors in the brain comes primarily from *in vitro* studies with brain tissue slices or synaptosomal preparations (Farnebo & Hamberger, 1971; Starke & Montel, 1973; Mulder *et al.*, 1978). Confirmation and expansion of *in vitro* results can now be obtained through *in vivo* microdialysis. The extracellular concentration of neurotransmitters, measured *in vivo* with brain microdialysis, often reflects active neuronal release (Ungerstedt, 1984; Westerink *et al.*, 1987). The extracellular NA measured in the cerebral cortex of the freely moving rat reflects calcium-dependent and tetrodotoxin-sensitive release (l'Heureux *et al.*, 1986; Van Veldhuizen *et al.*, 1990). *In vivo* it has been confirmed that α_2 -adrenoceptors control cerebral NA release (Dennis *et al.*, 1987; Abercrombie *et al.*, 1988; Itoh *et al.*, 1990). However, in these studies drugs were predominantly applied systemically.

In the present microdialysis study we used local drug administration, combining the advantages of *in vitro* studies, the possibility of comparing drug effects in cumulative dose-response studies (Frankhuijzen & Mulder, 1982), with the advantage of *in vivo* studies, the possibility of measuring

effects in an intact system. This method has some advantages over peripheral administration: (1) for each rat a cumulative concentration-response curve can be determined (*viz.* by successive addition to the perfusion fluid of increasing drug concentrations); (2) drugs can easily reach the target tissue (no blood brain barrier to pass); (3) effects of drugs are restricted to the area of application. We compared the effects of several locally applied α_2 - and α_1 -adrenoceptor agonists and antagonists on cortical NA overflow. In addition, we examined the effect of continuous activation of the α_2 -adrenoceptor. To investigate possible differences in passage of the drugs across the dialysis membrane, *in vitro* recovery of the drugs was also measured.

Methods

Animals

Male Wistar rats (180–250 g) were obtained from Harlan (Zeist, The Netherlands). Rats were housed individually in perspex cages (25 × 25 × 32 cm) during the microdialysis experiment. They had free access to food and water, and were subjected to a light/dark schedule of 12 h (lights on at 7 h 00 min). Testing took place during the light period.

Surgical procedure

Transcortical cannulae were made according to Imperato & Di Chiara (1984). The semipermeable membrane, with a dialysis zone of 9 mm, was of cellulose ester (*i.d.* 0.22 mm,

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o.d. 0.27 mm, mol wt. cut-off 10 kD; Cordis Dow Medical International). Under chloralhydrate anaesthesia (400 mg kg⁻¹, i.p.) a rat was mounted on a Kopf stereotaxic frame with the incisor bar set at -2.5 mm. The skull was exposed and holes were drilled on each side of the temporal bone. The cannula was inserted horizontally in the cortex (A 0, V -2.0 mm vs bregma). Both cannulae ends were bent upwards and cemented on the skull.

After the experiments the location of the cannula was verified. For this purpose the rats were decapitated, and brains were frozen for cryostat sectioning. Subsequently, sections were stained with thionine for histological evaluation.

Dialysis procedure

Experiments were carried out in freely moving rats the day after surgery. The transcortical cannula was perfused with Ringer solution (NaCl 154 mM, KCl 4 mM, CaCl₂ 2.2 mM, pH 6.2) at a flow rate of 3 µl min⁻¹. Cortical dialysate was collected in a 50 µl sample loop and injected automatically (Rheodyne 5701) on-line once every 20 min into a high performance liquid chromatography (h.p.l.c.) system.

Analysis of NA in dialysates

Dialysates were analysed for NA by reversed phase liquid chromatography at room temperature on a Supelcosil column (LC-18-DB, 250 × 4.6 mm, 5 µm) protected by a Supelguard column (LC-18-DB, 20 × 4.6 mm, 5 µm). The mobile phase was a citric acid (10.4 mM)/sodium acetate (61 mM) buffer (pH 5.1) with 7% methanol, 0.3 mM EDTA and varying concentrations (0.52–0.93 mM) of heptane sulphonic acid; the latter was used to move the NA peak to empty places in the chromatogram. The flow rate through the system was 0.8 ml min⁻¹ (Waters 510 or 590).

For electrochemical detection of NA we used an ESA Coulochem 5100A or 5200 dual electrode, cell model 5011, in a differential mode. At the first detector cell an oxidising potential between +350 and +400 mV was applied, while the second detector was set at a reducing potential between -250 and -400 mV. *In vivo* NA levels were compared with a standard NA solution in Ringer. The detection limit of NA was between 0.17 and 0.6 pg.

Drug treatment

Drug solutions were perfused after 5 steady basal NA samples (100 min) had been obtained. Each drug was administered in increasing concentrations and each concentration was perfused for 4 sampling periods of 20 min. The experiment was ended with 4 sampling periods under basal conditions (i.e. Ringer perfusion). For continuous activation of the α₂-adrenoceptor, the agonist UK-14,304 was perfused for about 3 h at a concentration of 10⁻⁶ M. For each drug, as well as for the continuous activation experiment, a group of 3 to 5 rats was used (in the case of clonidine 10⁻⁴ M, piperoxan and yohimbine 10⁻³ M only 2 measurements were available).

The following drugs were used: clonidine hydrochloride, (±)-piperoxan hydrochloride, (±)-methoxamine hydrochloride, (-)-phenylephrine hydrochloride (Sigma, St. Louis, U.S.A.), moxonidine base (BDF Beiersdorf, Hamburg, Germany), UK-14,304 tartrate (bromoxidine; Pfizer, Sandwich, UK), B-HT 933 dihydrochloride (azepevole; Boehringer, Ingelheim, Germany), (±)-idazoxan hydrochloride (Reckitt & Colman, Kingston upon Hull, UK), yohimbine hydrochloride (Janssen Chimica, Beerse, Belgium).

Data analysis

NA overflow was expressed as pg min⁻¹ ± s.e.mean without correction for recovery. Basal NA overflow for each animal was defined as the average of the 5 samples taken

immediately before drug application. In the illustrations drug-induced changes of NA overflow are expressed as percentages of this average basal level obtained in each individual rat. Statistical evaluation was done with absolute values. The first sampling period after each change in drug concentration was excluded from the analysis, since these results were likely to reflect the effects of a mixture of two concentrations. To compare differences among averages (basal versus drug-modulated overflow) the last three sampling periods of each drug concentration were averaged and compared by one-way analysis of variance (ANOVA) with repeated measures (level of significance α = 0.05; if n ≥ 3). Where appropriate, a *post-hoc* comparison of the means was made with the Newman-Keuls test. To compare the various drugs, the results were analysed with ANOVA (repeated measures) with Drug and Dose as the main factors.

In vitro recovery of drugs and NA

The recovery of drugs across the membrane was tested *in vitro* at room temperature by submersion of the cannula in a bath (10 ml) containing Ringer solution. The passage of drugs from the perfusion fluid to the bath was determined by perfusing Ringer solution containing drugs in known concentrations at 3 µl min⁻¹. After 20 min of equilibration, a 40 min sample of perfusate was collected for analysis of the drug. The cannula was perfused for 20 min with Ringer solution before another drug was tested.

The reverse situation, passage of drugs from the bath to the perfusion fluid, was also tested: Ringer solution was used as perfusion fluid and the membrane was now immersed in a 10 ml Ringer bath containing the drug (all drugs 10⁻⁴ M). As the cannulae were immersed in different drug solutions, the membranes were rinsed with Ringer solution before application of a new drug.

The recovery of NA was also determined as passage from the bath to the perfusion fluid, both in the absence and in the presence of drugs. The membrane was submerged in a NA solution (1 ng ml⁻¹) and Ringer or a 10⁻⁵ M drug solution was perfused. NA concentrations were determined as described above for the *in vivo* experiments.

Analysis of drugs was carried out on the same day by reversed-phase h.p.l.c. with u.v. detection (230 nm). The mobile phase was a sodium-phosphate buffer, containing 0.1% tri-ethylamine and adjusted to pH 3.0 with phosphoric acid. The concentration of acetonitrile differed for the various drugs: for B-HT 933 (azepevole) and phenylephrine 0%; for clonidine, moxonidine and UK-14,304 (bromoxidine) 10%; for methoxamine 15%; for idazoxan, piperoxan and yohimbine 25%; for prazosin 35% (Sigma, St. Louis, U.S.A.). The column was a Supelcosil column LC-18-DB (250 × 4.6 mm, 5 µm), the flow rate was 1.0 ml min⁻¹ (HP pump 1090) and the injection volume was 50 µl.

Recovery was expressed as a percentage and was calculated for passage across the membranes from the formula [(c_i-c_d)/c_i] × 100% when the drug was present in the perfusion medium, and (c_d/c_i) × 100% when the drug was present in the bath, with c_i being the initial drug concentration (in perfusion medium and bath, respectively) and c_d the drug concentration measured in perfusate or dialysate, respectively.

For each drug both directions of membrane passage were measured in triplicate in 3 membranes. A paired two-tailed Student's *t* test was used for each drug to test whether differences in recovery depend on the direction of membrane passage. Analysis of variance was applied to compare different drugs. The level of significance was α = 0.05.

Results

During the initial phase, basal NA overflow (5 steady basal levels) in cortical dialysates of freely moving rats was 0.250 ± 0.021 pg min⁻¹ (n = 42 rats). The basal levels in con-

trol rats may fluctuate during the measurement period (8–9 h), but no overall decrease or increase was seen ($n = 3$).

α_2 -Adrenoceptor agonists

Figure 1 exemplifies the concentration-dependent decrease in cortical NA overflow induced by α_2 -adrenoceptor agonists. The results with UK-14,304 clearly show that a new steady state NA level is achieved within one or two sampling periods after changing the perfusion medium. This new level remained constant in the following sampling periods apparently independent of the duration of the application, as shown in Figure 2 where one concentration of UK-14,304 was present for about 3 h. If after UK-14,304 Ringer solution was perfused again, NA levels did not recover to the pre-drug level within 80 min but returned to baseline gradually after 2–3 h ($n = 3$; data not shown).

The effects of UK-14,304 as well as of other α_2 -adrenoceptor agonists on cortical NA overflow are summarized in Figure 3. UK-14,304, clonidine, moxonidine and B-HT 933 all led to concentration-dependent reductions in NA overflow. UK-14,304 was the most potent α_2 -adrenoceptor agonist, with a half-maximal effect at 10^{-7} M. Whereas moxonidine and UK-14,304 reduced NA overflow to 10 to

Figure 1 Effect of UK-14,304 on cortical noradrenaline (NA) overflow. UK-14,304 was dissolved in Ringer solution and each concentration was applied for 80 min. Columns represent means $\pm$ s.e.mean expressed as a percentage of basal overflow (Ringer perfusion). Basal NA overflow was 0.143 ± 0.020 pg min $^{-1}$ ($n = 5$ rats).

Figure 2 Effect of continuous perfusion with UK-14,304 on cortical noradrenaline (NA) overflow. The drug was dissolved in Ringer solution and a concentration of 10^{-6} M UK-14,304 was applied for 3 h. Columns represent means $\pm$ s.e.mean expressed as a percentage of basal overflow. Basal NA overflow was 0.381 ± 0.094 pg min $^{-1}$ ($n = 3$).

Figure 3 Effect of α_2 -adrenoceptor agonists on cortical noradrenaline (NA) overflow. The drugs were dissolved in Ringer solution and each concentration was applied for 80 min. The points represent means $\pm$ s.e.mean of the last 60 min of infusion obtained in 3 to 5 rats per drug. Basal NA overflow was 0.276 ± 0.074 pg min $^{-1}$, ($n = 3$) (○) ($n = 2$ at 10^{-4} M (dotted line)); for moxonidine 0.232 ± 0.020 pg min $^{-1}$, $n = 3$ (●); for UK-14,304 0.143 ± 0.020 pg min $^{-1}$, $n = 5$ (△); for B-HT 933 0.216 ± 0.037 pg min $^{-1}$, $n = 3$ (▲). Drug-evoked differences from basal level are statistically significant for UK-14,304 from 10^{-7} M onwards, for clonidine and moxonidine from 10^{-6} M onwards and for B-HT 933 from 10^{-4} M onwards (ANOVA followed by post-hoc Newman-Keuls).

Figure 4 Effect of idazoxan on cortical noradrenaline (NA) overflow. Idazoxan was dissolved in Ringer solution and each concentration was applied for 80 min. Columns represent means $\pm$ s.e.mean expressed as a percentage of basal overflow. Basal NA overflow was 0.212 ± 0.050 pg min $^{-1}$ ($n = 3$).

15%, both at 10^{-5} M, the effect of 10^{-4} M clonidine or B-HT 933 was a reduction to no more than 30% and 47%, respectively. Moxonidine and clonidine were about equipotent but had different maximal effects, while B-HT 933 was the least potent and least effective agonist.

α_2 -Adrenoceptor antagonists

Local administration of the α_2 -adrenoceptor antagonist, idazoxan, caused a concentration-dependent increase in extracellular NA (Figure 4). Again, a new equilibrium was reached after one or two sampling periods for each concentration. Ringer perfusion after drug treatment led to a rapid return of NA concentrations to basal levels.

The effects of the α_2 -adrenoceptor antagonists on NA overflow are depicted in Figure 5. A concentration-dependent significant increase in NA overflow also occurred during perfusion with piperoxan, but this response was less marked than the response to idazoxan. In contrast, yohimbine failed to have an effect.

Figure 5 Effect of α_2 -adrenoceptor antagonists on cortical noradrenaline (NA) overflow. The drugs were dissolved in Ringer solution and each concentration was applied for 80 min. The points represent means $\pm$ s.e.mean of the last 60 min of infusion obtained in 3 rats per drug (for piperoxan and yohimbine $n = 2$ at 10^{-3} M (dotted line)). Basal NA overflow was for idazoxan 0.212 ± 0.050 pg min $^{-1}$ (O); for piperoxan 0.389 ± 0.053 pg min $^{-1}$ (Δ) and for yohimbine 0.227 ± 0.074 pg min $^{-1}$ ($\square$). Drug-evoked differences from basal level are statistically significant for idazoxan from 2×10^{-5} M onwards and for piperoxan from 10^{-4} M onwards; for yohimbine no effect was found (ANOVA followed by post-hoc Newman-Keuls).

α_1 -Adrenoceptor ligands

Local administration of the α_1 -adrenoceptor agonists, methoxamine and phenylephrine, had no effect on NA overflow at low concentrations ($n = 3$, results not shown); concentrations of 10^{-3} M increased NA to 225% (phenylephrine; $n = 1$) and 300% (methoxamine; $n = 2$), respectively.

In vitro recovery of drugs and NA

Calibration curves of the drugs were linear in the concentration-range that was used for the *in vivo* experiments: 10^{-6} – 10^{-3} M for agonists and 10^{-6} – 10^{-4} M for antagonists.

With the exception of prazosin, *in vitro* recovery was similar, about 10–20%, in either direction across the mem-

brane and showed no significant differences between drugs (Table 1). The α_1 -adrenoceptor antagonist prazosin adhered so much to the membrane and the perfusion tubing that only 30% of the initial perfusion concentration (10^{-4} M) was detected in the perfusate. In the reverse situation, when the membrane was placed in 10^{-4} M prazosin, the dialysate contained only 2%.

The passage of NA from the bath through the dialysis membrane into the Ringer solution (recovery) was $17.4 \pm 0.3\%$ ($n = 3$ membranes in duplicate). The recovery of NA was not influenced by the presence of any of the drugs (10^{-5} M) in the perfusion fluid.

Discussion

The present study shows that it is possible to determine cumulative concentration-response curves and, hence, potencies of drugs *in vivo* by using brain microdialysis combined with local administration of drugs via the perfusion fluid. This is the first comparison of the effects of several locally applied drugs selective for the α_2 -adrenoceptor on central NA overflow *in vivo*. The experimental set-up proved to be satisfactory. Within 20 to 40 min after the start of drug perfusion, the overflow of NA reached a new steady state and always returned to baseline values after drug treatment. Why baseline values were obtained more rapidly after the testing of antagonists than after agonists is not clear.

The NA overflow which we determined in the cortex represents a release related to basal neuronal activity. The local effects of the α_2 -adrenoceptor antagonists show that under basal conditions, at the level of the nerve terminal, the NA overflow was under tonic autoinhibitory control. The local effects of exogenous α_2 -adrenoceptor agonists show that NA overflow could be reduced to 10–15% of baseline values. This is similar to the level that is reached when overflow is reduced by addition of tetrodotoxin or by omission of calcium from the Ringer solution (Van Veldhuizen *et al.*, 1990), and it is probably the maximal response to be obtained with the present experimental set-up.

The actual value of basal NA overflow we determined in the cerebral cortex was 0.25 pg min $^{-1}$. This is similar to our previous measurements (Van Veldhuizen *et al.*, 1990), but lower than the levels reported by Dennis *et al.* (1987) and l'Heureux *et al.* (1986), who used a similar set-up. These differences are still common in microdialysis literature and are likely to be caused by differences in experimental conditions.

In vitro recovery

To compare drug effects one has to know whether the drugs pass the membrane to a similar extent. Our measurements on *in vitro* recovery show that all drugs had about the same recovery and that recovery was similar in both directions across the dialysis membrane. An exception was prazosin, which adhered so much to the membrane and perfusion tubing that it was not administered *in vivo*. For the other drugs recovery across the membrane was 10–20%, which means that the concentrations of the drugs in the brain tissue are probably 5 to 10 fold lower than in the perfusion fluid. Regrettably, *in vitro* recovery never exactly predicts the real concentration *in vivo* (Benveniste & Hüttemeier, 1989). When it is considered that recovery increases with increasing temperature, the 10–20% recovery agrees with the 25% recovery that Dennis *et al.* (1987) reported in an *in vivo* experiment with radiolabelled [3 H]-idazoxan. From the similar recovery of the drugs, and the fact that the recovery of NA was not influenced by any of the drugs, we conclude that observed differences in drug effects on NA overflow solely reflect differences in interaction between drugs and brain tissue.

Table 1 *In vitro* recovery of drugs

	Drug in perfusion fluid [($c_i - c_d$)/ c_i] $\times 100\%$	Drug in bath (c_d/c_i) $\times 100\%$
α_1-Adrenoceptor agonists	10^{-5} M	10^{-4} M
Clonidine	15.7 ± 2.2	16.0 ± 1.3
Moxonidine	11.0 ± 0.5	10.3 ± 1.3
UK-14,304	19.5 ± 2.5	13.6 ± 1.3
B-HT 933	13.5 ± 1.9^b	12.0 ± 0.9
α_2-Adrenoceptor antagonists	10^{-5} M	10^{-4} M
Idazoxan	12.7 ± 0.7	$12.0/15.2^a$
Piperoxan	ND	$9.3/14.7^a$
Yohimbine	13.2 ± 1.5	$9.4/11.2^a$
α_1-Adrenoceptor ligands	10^{-4} M	10^{-4} M
Phenylephrine	11.0 ± 1.9	ND
Methoxamine	11.0 ± 1.5	ND
Prazosin	$70.6 \pm 0.5^*$	2.4 ± 0.3

Recovery of drugs was determined in 3 membranes. Means $\pm$ s.e.mean are given in percentages. Drugs were measured in the initial standard solution (perfusion fluid or bath), resulting in c_i , and in the perfusate or dialysate, resulting in c_d .

^a $n = 2$ measurements; ^b 10^{-4} M; ND, not determined.

* $P < 0.05$, paired Student's *t* test compared to drug in bath.

Effects of locally applied drugs

In the present study both moxonidine and UK-14,304 (bromoxidine) were more effective in reducing NA overflow than clonidine or B-HT 933 (azepexole). These *in vivo* results confirm earlier *in vitro* findings that UK-14,304 and moxonidine are full α_2 -adrenoceptor agonists (Grant & Scrutton, 1980; Armah, 1988), in contrast to the partial agonism which has been described for clonidine (Medgett *et al.*, 1978). A previous *in vitro* study with B-HT 933, however, did not predict the restricted effectiveness of this compound (Cichini & Singer, 1987).

The α_2 -adrenoceptor antagonists caused a concentration-dependent increase in NA overflow, with idazoxan being more potent than piperoxan. The absence of an effect on NA overflow by yohimbine is discussed below.

The effect of the α_1 -adrenoceptor agonists, an increase in NA overflow, suggests that NA overflow is also modulated by the α_1 -adrenoceptor. However, this effect was only found at high concentrations. In some tissues activation of presynaptic α_1 -adrenoceptors is thought to result in an inhibition of NA release (Murphy & Majewski, 1989). On the other hand, some α_1 -adrenoceptor agonists may release NA by a tyramine-like mechanism (Vizi *et al.*, 1986).

While the difference in potency between idazoxan and piperoxan is in agreement with literature data (Doxey *et al.*, 1983; Baron & Siegel, 1990; Mermet *et al.*, 1990) and our own data on [3 H]-RX821002 binding to central α_2 -adrenoceptors (Boer *et al.*, 1993), the comparison of the results obtained with agonists with literature data presents some problems. The evoked *in vitro* release of [3 H]-NA from rat cortical slices was reduced less potently by moxonidine than by clonidine (pIC_{35} 6.9 vs 7.7; Schlicker *et al.*, 1990), and UK-14,304 and clonidine were about equipotent (Ong *et al.*, 1991), in contrast to our results. This order in *in vitro* potency was confirmed by binding studies (clonidine $\approx$ UK-14,304 > moxonidine > B-HT 933; Boer *et al.*, 1993), whereas *in vivo* UK,14,304 > moxonidine $\approx$ clonidine > B-HT 933 was found. Thus, in relation to the other drugs, clonidine appeared to be more potent *in vitro* than *in vivo*. Part of these discrepancies may be due to the fact that the experimental design in *in vitro* release studies is basically different from that *in vivo*; high potassium concentrations or repetitive electric pulses are used in a standardized way to stimulate NA release, and measurements are made in a damaged biological system. In contrast, *in vivo* non-stimulated NA overflow can be measured in an intact biological system.

Local versus systemic administration

The concentration profile of the locally administered compound in the surrounding tissue is usually unknown. However, continuous application of UK-14,304 resulted in a stable level of NA within 20–40 min, suggesting that there is no accumulation of drug in the tissue surrounding the membrane. Therefore the effect of the previous drug concentration will be negligible in cumulative concentration-response curves (Frankhuysen & Mulder, 1982).

Systemically injected α_2 -adrenoceptor drugs can affect transmitter overflow by actions on receptors located on the nerve terminal and on somatodendritic autoreceptors located in the cell body region. Through local perfusion of drugs the contribution of somatodendritic receptors can be excluded. Comparison of the effects of drugs administered in one of both ways on the overflow at the level of the nerve terminal can reveal the contribution of each receptor population (Westerink *et al.*, 1990).

Local administration of 10^{-5} M clonidine caused a 60% reduction in cortical NA overflow, similar to the 50–60% reduction after systemic administration of 0.3 mg kg $^{-1}$, i.p.,

reported in the cortex by l'Heureux *et al.* (1986) and in the hippocampus by Abercrombie *et al.* (1988). The clonidine concentration in brain tissue after the systemic administration of 0.4 mg kg $^{-1}$ clonidine (Nazarali *et al.*, 1986) was 2×10^{-6} M, which is in the same range as that around our membranes when corrected for recovery. Thus, in the cortex the effect of 0.3 mg kg $^{-1}$, i.p. clonidine seems comparable with the effect of 10^{-5} M perfused locally. This might indicate that activation of somatodendritic α_2 -autoreceptors on NA cell bodies does not add to the local effect of clonidine.

Locally perfused 10^{-4} M idazoxan caused an increase in cortical NA overflow to 300%, which is comparable to the 250% increase after local perfusion of 10^{-4} M idazoxan reported by Dennis *et al.* (1987) and to the 250–300% increase after systemic administration of 20 mg kg $^{-1}$, i.p., idazoxan (l'Heureux *et al.*, 1986; Dennis *et al.*, 1987). Again, both central and peripheral drug administration resulted in comparable effects on cortical NA overflow.

However, for yohimbine the difference between local infusion and systemic administration is quite profound. Yohimbine has been shown to produce increases in NA release in most *in vitro* and *in vivo* studies (e.g. Taube *et al.*, 1977; Szemerédi *et al.*, 1991; reviews by Langer, 1981; Goldberg & Robertson, 1983; Starke *et al.*, 1989), but had no significant effect in the present *in vivo* dialysis study. This lack of effect must be the result of the local administration, since systemic administration (5.0 mg kg $^{-1}$, i.p.) increased NA overflow to 200–230% in the cortex (own unpublished data) and hippocampus (Abercrombie *et al.*, 1988). This discrepancy is not due to a hampered passage across the dialysis membrane or to interference with the passage of NA. Nor is it due to the non-imidazoline character of yohimbine as compared to idazoxan (Bousquet *et al.*, 1984; Tibiriça *et al.*, 1991). Drugs with an imidazoline structure and affinity for the α_2 -adrenoceptor (such as clonidine, UK-14,304, moxonidine and idazoxan) can influence NA overflow via an imidazoline receptor. Two findings suggest that α_2 -adrenoceptors but not imidazoline receptors are involved in the response measured. First, we showed that the non-imidazolines B-HT 933 and piperoxan affected NA overflow in concentrations relevant for interaction with α_2 -adrenoceptors. Second, idazoxan increased NA overflow, whereas it has been shown to inhibit [3 H]-NA release via an imidazoline receptor after α_2 -adrenoceptor blockade with rauwolscine (Göthert & Molderings, 1991). Apparently, inhibition of somatodendritic autoreceptors on NA neurones by yohimbine *in vivo* results in increased NA overflow in terminal regions, whereas local administration does not. However, there may be alternative explanations since yohimbine has, besides its well documented and unequivocal α_2 -adrenoceptor antagonistic properties (see above), a variety of other effects (Goldberg & Robertson, 1983). For instance, local anaesthetic actions in central neurones have been reported to result in an inhibitory effect on NA release (Hagan & Hughes, 1986). It is not clear, however, why these effects would be apparent only after local administration, even of low concentrations. Another explanation could be that upon local administration the inhibitory effects of yohimbine on the α_2 -adrenoceptor are balanced by an action on other receptors, e.g. 5-hydroxytryptamine receptors for which yohimbine has significant affinity (Goldberg & Robertson, 1983; Winter & Rabin, 1992).

We want to thank H. Stoffels for drawing the illustrations, Prof. Dr D.F. Swaab for critically reading the manuscript and A. Janssen for his linguistic comments. The work was supported by the Van den Houten Foundation.

Moxonidine, UK-14,304, B-HT 933 and idazoxan were generous gifts of BDF Beiersdorf, Pfizer, Boehringer and Reckitt & Colman, respectively.

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(Received January 14, 1993)

Revised February 15, 1993

Accepted February 23, 1993)