British Journal of Pharmacology (1995) 114, 1717–1723

Contrasting effects of the imidazol(in)e α_2-adrenoceptor agonists, medetomidine, clonidine and UK 14,304 on extraneuronal levels of noradrenaline in the rat frontal cortex: evaluation using *in vivo* microdialysis and synaptosomal uptake studies

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1 *In vivo* microdialysis in halothane-anaesthetized rats and synaptosomal [^{3}H]-noradrenaline uptake studies *in vitro* were used to evaluate the effects of imidazole (medetomidine) and imidazoline (clonidine and UK 14,304) α_2-adrenoceptor agonists on extraneuronal levels of noradrenaline in the frontal cortex.

2 Levels of noradrenaline in the dialysate were increased by a depolarizing concentration of K^+ (60 mM for 20 min) and substantially attenuated by reducing Ca^{2+} supply in the perfusate. These results suggest that spontaneous efflux of noradrenaline in the cortex is regulated predominantly by cation-dependent exocytotic mechanisms.

3 At a low perfusion concentration (0.5 μM), medetomidine, clonidine and UK 14,304 all reduced the level of noradrenaline in cortical dialysates. Continuous perfusion of the selective α_2-adrenoceptor antagonist, atipamezole (0.5 μM) caused a sustained increase in noradrenaline efflux and reversed the inhibitory effects of medetomidine. All these changes are consistent with drug actions at presynaptic α_2-adrenoceptors.

4 Higher concentrations of medetomidine (5–50 μM), but not clonidine or UK 14,304, evoked a non-desensitizing increase in noradrenaline efflux. This effect was not antagonized by 0.5 μM atipamezole.

5 The tricyclic noradrenaline reuptake inhibitor, desmethylimipramine (0.5–50 μM), increased noradrenaline efflux in a concentration-dependent manner.

6 The specific uptake of [^{3}H]-noradrenaline into cortical synaptosomes was inhibited by medetomidine and desmethylimipramine with IC_{50} values of approximately 7 μM and 8 μM respectively. Neither clonidine nor UK 14,304 inhibited [^{3}H]-noradrenaline uptake.

7 These results indicate that micromolar concentrations of the selective α_2-adrenoceptor agonist, medetomidine, can augment extraneuronal levels of noradrenaline in the rat frontal cortex; this effect seems to involve an inhibition of noradrenaline reuptake rather than an action at α_2-adrenoceptors.

Keywords: Atipamezole; clonidine; cortical synaptosomes; desmethylimipramine; frontal cortex; medetomidine; microdialysis; UK 14,304

Introduction

It is widely accepted that presynaptic α_2-adrenoceptors modulate noradrenaline release in the brain. Evidence to support this view stems from experiments *in vitro* studying transmitter release from synaptosomes (de Langen *et al.*, 1979; Maura *et al.*, 1992) and brain slices (Reichenbacher *et al.*, 1982; Raiteri *et al.*, 1992), and from experiments using *in vivo* microdialysis (L'Heureux *et al.*, 1986; Thomas & Holman, 1991; van Veldhuizen *et al.*, 1993). In all of these studies, noradrenaline efflux was reduced by α_2-adrenoceptor agonists and increased by α_2-adrenoceptor antagonists.

The imidazole derivative, (±)-4-[1-(2,3-dimethylphenyl) ethyl]-1H-imidazole (medetomidine), is a potent α_2-adrenoceptor agonist with an α_2/α_1 selectivity ratio of over 1600 (Virtanen *et al.*, 1988). In recent experiments, we used *in vivo* microdialysis to test the effects of this compound on noradrenaline efflux in the rat frontal cortex. The drug was administered directly into the terminal field *via* the dialysis probe. We found that the effects of medetomidine were strongly concentration-dependent. At a low perfusion concentration (0.5 μM), medetomidine reduced noradrenaline levels in the dialysate but higher concentrations evoked a non-desensitizing increase in noradrenaline efflux. These preliminary findings have been reported to the British Pharmacological Society (Dalley & Stanford, 1994). The aim of the present study was to eliminate the possibility that this increase in noradrenaline efflux involves α_2-adrenoceptors. To achieve this, we first tested whether the increase could be prevented by the selective α_2-adrenoceptor antagonist, atipamezole. In further studies the effects of medetomidine on noradrenaline efflux were compared with those of the imidazoline α_2-adrenoceptor agonists, clonidine and 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine (UK 14,304).

One limitation of *in vivo* microdialysis is that levels of noradrenaline in the dialysate reflect the net influence of both neurotransmitter release from the nerve terminals and its neuronal and non-neuronal reuptake from the extracellular fluid. Spontaneous changes in noradrenaline efflux are highly Ca^{2+}-dependent (L'Heureux *et al.*, 1986; van Veldhuizen *et al.*, 1990), suggesting that it is mainly transmitter release which determines the levels of noradrenaline in the dialysate under basal conditions. However, drug-induced changes in noradrenaline efflux could reflect changes in release and/or

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reuptake. For this reason, further studies were carried out to compare the effects of medetomidine, clonidine and UK 14,304 on synaptosomal uptake of [^{3}H]-noradrenaline, using the potent noradrenaline reuptake inhibitor, desmethylimipramine, as an active control. We show that, whereas medetomidine and desmethylimipramine both increase noradrenaline efflux in the rat frontal cortex and inhibit cortical synaptosomal uptake of [^{3}H]-noradrenaline *in vitro*, neither of these effects is apparent with clonidine or UK 14,304.

Methods

Intracerebral microdialysis

Animals Male Sprague-Dawley rats (250–310 g) were obtained either from University College London (U.K.), or from Charles River (U.K.). Rats were maintained in groups of 4 at 21–24°C with a light/dark schedule of 12 h (lights on at 08 h 00 min) and allowed access to food and water *ad libitum*.

Surgical procedures Intracerebral microdialysis probes were constructed with a 5 mm conducting zone of semipermeable membrane (i.d. 200 μm, o.d. 300 μm, mol.wt. cut-off 20 kD; Filtral 12, Hospal Industrie, France) essentially as described by Sandberg *et al.* (1986). Under halothane anaesthesia, a tracheal cannula was inserted and used to deliver freely inspired halothane (1–1.5%) mixed in 95% O_2 and 5% CO_2 at 400 ml min^{-1}. The core body temperature was maintained at 37°C with a thermostatically controlled heating blanket in conjunction with a rectal probe (Harvard Instruments).

Rats were secured in a Kopf stereotaxic frame and the skull exposed to reveal bregma. After a craniotomy and removal of the dural membrane, a Ringer-primed microdialysis probe was inserted vertically into either the left or right frontal cortex (A 3.5, L 1.5, V 5.5 mm) according to published stereotaxic coordinates (Paxinos & Watson, 1986). At the conclusion of the experiment the location of the probe was verified histologically. For this purpose the brain was removed, fixed in 10% formaldehyde in normal saline, cryostatically sectioned, and examined under a low-power binocular microscope.

Dialysis procedure The dialysis probe was perfused with Ringer solution (composition mM: NaCl 150, KCl 4, $CaCl_2$ 1.2, pH 6.1) at 1.0 μl min^{-1}. *In vitro* calibration studies, carried out before experimentation, revealed that the relative recovery of noradrenaline was approximately 35% at 1.0 μl min^{-1}. Cortical dialysates were collected every 20 min into 5 μl 0.01 M perchloric acid. Experiments commenced, at the same time each day, 2.5 h after probe implantation.

Analysis of noradrenaline in dialysates Dialysates were analysed for noradrenaline content by reverse-phase high pressure liquid chromatography with electrochemical detection. Noradrenaline was separated at room temperature (maintained at 24°C) on a Hypersil ODS 5 μm cartridge column (25 cm × 4.6 mm; Fisons) protected by an Aquapore (7 μm) guard column (30 mm × 4.6 mm; Applied Biosystems). The filtered and degassed mobile phase, which contained sodium dihydrogen orthophosphate (83 mM), sodium (−)-octanesulphonic acid (2.77 mM), EDTA (0.85 mM), methanol (12%), and adjusted to pH 3.4 with orthophosphoric acid, was recycled at 1.275 ml min^{-1} (ESA model 580 solvent delivery module). Pressure fluctuations were buffered with a pulse dampener.

Electrochemical detection of noradrenaline was achieved with a dual electrode Coulochem II system (ESA) in redox mode. At the first analytical electrode a reducing potential of −275 mV was applied and, at the second (noradrenaline detecting) electrode, an oxidising potential of +275 mV was applied. A pre-injection guard cell set at +325 mV was used to condition the mobile phase. Standards and dialysates were injected manually into the loading port (Rheodyne 7125) with a loop volume of 20 μl. Chromatograms were relayed to an integrator (Spectra-Physics) and peak height used to quantitate noradrenaline levels. The detection limit for noradrenaline was approximately 2 fmol.

Drug treatment Drug solutions were perfused after 3 stable basal dialysates had been collected. Each drug was administered in increasing concentrations (0.5 μM, 5.0 μM and 50 μM) and each concentration was perfused for 3 sampling periods of 20 min. Atipamezole (0.5 μM) was continuously perfused, either alone, or in combination with increasing concentrations of medetomidine. In studies designed to confirm a neuronal origin of noradrenaline, either KCl was added to the perfusate (final concentration 60 mM) for 20 min, or $CaCl_2$ removed for 80 min. In these experiments the tonicity of the perfusate was maintained by adjusting the concentration of NaCl. The dead volume between the drug injection switch and the dialysis probe outlet was 10–15 μl. The following drugs were used: medetomidine hydrochloride (Orion-Farmos, Finland), clonidine hydrochloride (Boehringer Ingelheim, Germany), UK 14,304-18 (Pfizer, U.K.), atipamezole hydrochloride (Norden Laboratories), desmethylimipramine hydrochloride (Sigma).

Uptake of (−)-[^{3}H]-noradrenaline into cortical synaptosomes

Preparation of synaptosomes Male Sprague-Dawley rats (175–225 g) were killed by stunning and cervical dislocation and their brains removed. Crude synaptosomes were prepared essentially as described by Redfern *et al.* (1993). The cerebral cortex was dissected on ice and gently homogenized in 2 ml of 0.32 M sucrose in a glass homogenizer fitted with a Teflon pestle. The homogenizer was washed twice with 2 ml sucrose and the wash added to the homogenate which was then centrifuged at 650 *g* for 10 min at 4°C. The supernatant was then spun at 12,000 *g* for 20 min at 4°C and the final pellet resuspended in 0.32 M sucrose at a concentration of 1 g wet weight of the original tissue per 2 ml sucrose.

Measurement of uptake of (−)-[^{3}H]-noradrenaline To 100 μl of the synaptosomal suspension were added 200 μl of Tris-Krebs buffer (containing mM: NaCl 136, KCl 5, $MgCl_2$ 12, $CaCl_2$ 2.5, (+)-glucose 10, (−)-ascorbate 1, Tris base 20, pargyline hydrochloride 0.25). The buffer was gassed with 95% O_2 and 5% CO_2 for 30 min and the pH adjusted to 7.4 with 0.1 M HCl before use; 100 μl aliquots containing dissolved test compounds were added to give a final concentration of 0.5 μM, 5 μM or 50 μM. Buffer (100 μl) was added to remaining samples to standardize the total volume. All samples were prepared in duplicate and preincubated for 3 min at either 37°C or 4°C; the latter served as a blank for non-specific uptake. The assay was started by addition of 100 μl [^{3}H]-noradrenaline to all samples to give a final concentration of 50 nM. The [^{3}H]-noradrenaline solution was prepared from a stock of (−)-[ring-2,5,6-^{3}H]-noradrenaline of specific activity 71.7 Ci mmol^{-1}, diluted 10 fold with unlabelled (−)-noradrenaline. After 3 min the incubation was terminated by filtration of the samples over Whatman GF/B filter discs. These were washed twice with 2 ml Tris-Krebs buffer and counted for tritium content in 9 ml scintillation fluid (Packard 'Ultima Gold'). The d.p.m. in each sample was generated from a standard quench curve.

The [^{3}H]-noradrenaline content of the samples is expressed as pmol mg^{-1} protein. Active uptake of [^{3}H]-noradrenaline was calculated as the difference in uptake at 37°C and 4°C; this typically accounted for 80% of the total. The protein content of the samples was measured in duplicate aliquots using the method of Lowry *et al.* (1951).

Statistical analysis The noradrenaline content of the dialysates is expressed as fmol 20 μl^{-1} ± s.e.mean, without correction for recovery. Statistical analyses were carried out on the raw data. Analysis of the effect of each drug treatment on noradrenaline efflux was carried out on orthonormalised data (*i.e.* on orthogonal contrasts: Norusis, 1990), with time as a within-subjects factor, using the MANOVA facility on SPSS PC$^+$. Averaged *F*-tests were used to assess the level of statistical significance of any changes in noradrenaline efflux. Comparison of drug effects at different doses used MANOVA, with time and drug concentration as within subjects factors. Again averaged *F*-tests were used to assess the level of significance when multivariate tests indicated significant changes in main effects of time or drug concentration. When these indicated statistically significant main effects, MANOVA was used on relevant clusters of data to compare the effects of specific drug concentrations. Again, concentration and time were used as within-subjects factors. A significant multivariate test was the criterion for using averaged *F*-tests to assess the level of significance of any differences.

Effects of individual drugs on [^{3}H]-noradrenaline uptake were evaluated from orthonormalised raw data using the one-way ANOVA facility on SPSS PC$^+$. When the ANOVA indicated a significant drug effect, a *post hoc* Tukey multiple range test was used to test for the significance of differences between means; the criterion for significance was set at $P<0.05$. Two-way ANOVA was used to compare the effects of different drugs on [^{3}H]-noradrenaline uptake with drug and concentration as the main factors.

Results

Effects of increased [K^+] or reduced [Ca^{2+}] on noradrenaline levels in cortical dialysates

Typical chromatograms showing the elution times, separation and content of noradrenaline, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindole-3-acetic acid in an external standard and basal cortical dialysate are given in Figure 1. Spontaneous efflux of noradrenaline in the frontal cortex was approximately 20 fmol 20 μl^{-1}. This remained constant throughout the entire time course of the experiment: a sequence of 14 consecutive samples showed no significant change with respect to time ('extended basals' $F=0.89$; d.f. 14,56; $P=0.578$) (Figure 2).

In experiments designed to assess whether the basal level of noradrenaline in the dialysate was derived from exocytotic release of transmitter, its sensitivity to the ionic composition of the perfusion medium was assessed (Figure 2). Perfusion of K^+ (60 mM) for 20 min increased noradrenaline efflux by 82%; this was statistically significant compared with the noradrenaline efflux in three basal dialysates.

Removal of Ca^{2+} from the perfusion medium produced a significant reduction (by 66%) in the dialysate concentration of noradrenaline. The basal levels of noradrenaline were restored when Ca^{2+} was reintroduced in the perfusion medium. Collectively, these results confirm the mainly neuronal origin of noradrenaline in cortical dialysates.

Effects of medetomidine, UK 14,304, clonidine and desmethylimipramine on noradrenaline levels in cortical dialysates

When medetomidine was perfused *via* the dialysis probe, there were main effects of drug concentration ($F=37.83$; d.f. 3,12; $P<0.001$) and time ($F=53.25$; d.f. 2,8; $P<0.001$) as well as a concentration × time interaction ($F=31.11$; d.f. 6,24; $P<0.001$) (Figure 3). Specific comparisons exposed a reduction in noradrenaline efflux induced by 0.5 μM medetomidine with respect to basal efflux. This reached a maximum reduction of 25% after 1 h, but was not statistically significant ($F=1.41$; d.f. 1,4; $P=0.301$). In contrast, increasing the concentration of medetomidine to 5 μM increased noradrenaline efflux (Figure 3). Overall, levels of noradrenaline during perfusion with 5 μM medetomidine were significantly higher than those measured after 0.5 μM medetomidine ($F=10.86$; d.f. 1,4; $P=0.03$) but were not significantly different from basal levels ($F=0.89$; d.f. 1,4; $P=0.398$). Increasing the concentration of medetomidine to

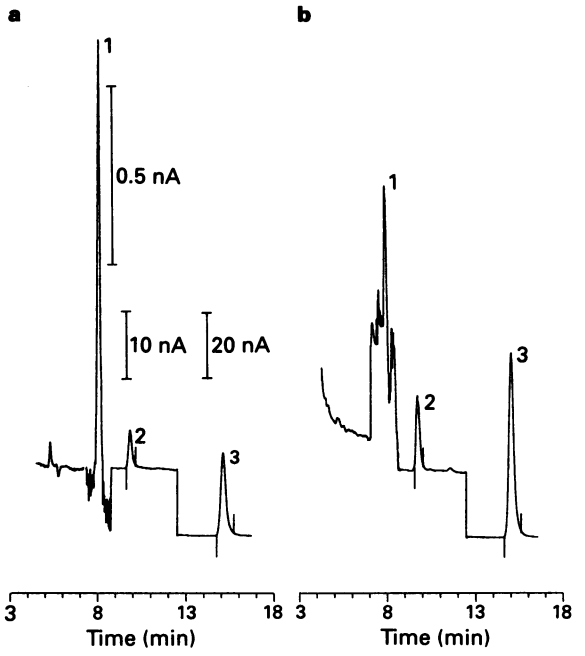

Figure 1 Representative chromatograms of (a) an external standard containing 50 fmol noradrenaline (1), 500 fmol, 3,4-dihydroxyphenylacetic acid (2), and 2 pmol 5-hydroxyindole-3-acetic acid (3), and (b) a dialysate from the frontal cortex, during Ringer perfusion which contains 22.7 fmol noradrenaline.

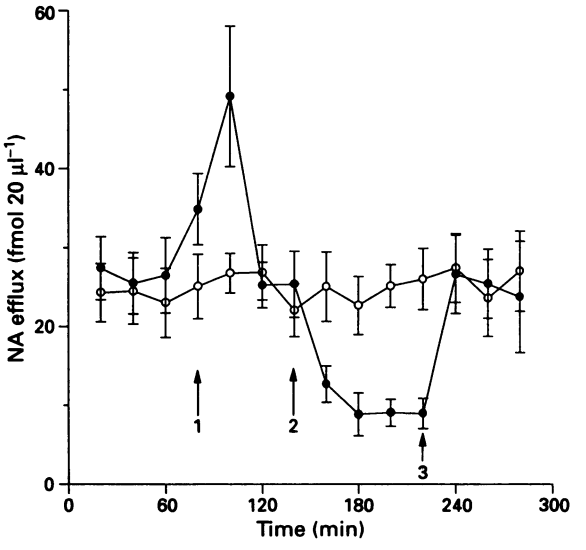

Figure 2 Extended time course of basal noradrenaline (NA) efflux in dialysates of the rat frontal cortex (○) and changes in noradrenaline efflux evoked by modification of the cation composition of the perfusion medium (●). Across all time points, there was a significant main effect of cation treatment ($F=19.69$; d.f. 3,12; $P<0.001$). K^+ (60 mM for 20 min) added at (1), significantly increased noradrenaline efflux ($F=48.95$ d.f. 1,4 $P=0.002$) relative to the three basal samples. Ca^{2+} removal for 80 min (2) significantly reduced noradrenaline efflux ($F=16.26$ d.f. 1,4 $P=0.016$) which returned to basal levels when Ca^{2+} was reinstated at 3 ($F=0.58$ d.f. 1,4 $P=0.58$).

50 μM produced a greater increase in noradrenaline efflux: at this concentration, noradrenaline efflux was significantly greater than both basal efflux ($F = 29.68$; d.f. 1,4; $P = 0.006$) and that induced by 5 μM medetomidine ($F = 170.1$; d.f. 1,4; $P < 0.001$).

Perfusion of clonidine *via* the dialysis probe reduced noradrenaline efflux with main effects of concentration ($F = 121.9$; d.f. 3,9; $P < 0.001$) and time ($F = 11.91$; d.f. 2,6; $P < 0.008$) (Figure 3). At 0.5 μM clonidine, noradrenaline efflux was reduced when compared with that in the basal samples ($F = 121.85$; d.f. 1,3; $P < 0.003$) (Figure 3). This reached a maximum of 36% after 1 h. There was a further reduction in noradrenaline levels in the dialysate when the concentration of clonidine was increased to 5 μM; this reduction was significantly greater than that caused by 0.5 μM clonidine ($F = 162.09$; d.f. 1,3; $P < 0.001$). Increasing the concentration of clonidine to 50 μM did not further reduce noradrenaline efflux ($F = 0.69$; d.f. 1,3; $P = 0.467$ *cf.* efflux with 5 μM clonidine).

UK 14,304 also reduced noradrenaline efflux (Figure 3) but main effects of concentration (multivariate analysis: $P = 0.106$) and time (multivariate analysis: $P = 0.095$) across all samples were not statistically significant. This is because the effects of this drug on noradrenaline efflux rapidly attained maximal inhibition at 0.5 μM and subsequent samples showed no appreciable change. Notwithstanding a reduction in efflux of 37% after 1 h, this reduction was not statistically significant when compared with basal efflux ($F = 6.27$; d.f. 1,3; $P = 0.087$) (Figure 3). However, increasing the concentration of UK 14,304 to 5 μM reduced noradrenaline efflux by a maximum of 42%; this reduction was significant when compared with basal efflux ($F = 27.19$; d.f. 1,3; $P = 0.014$). There was no further reduction in noradrenaline levels when the concentration of UK 14,304 was increased to 50 μM ($F = 1.92$; d.f. 1,3; $P = 0.26$ *cf.* 5 μM UK 14,304).

Perfusion of 0.5–50 μM desmethylimipramine caused a progressive increase in levels of noradrenaline in the dialysate (Figure 3). Whereas 0.5 μM desmethylimipramine had no effect on noradrenaline levels in the dialysate, both 5 μM ($F = 8.67$; d.f. 1,4; $P = 0.042$) and 50 μM ($F = 13.57$; d.f. 1,4; $P = 0.021$) drug concentrations caused a significant increase with respect to basal efflux. Moreover, the increase induced by 50 μM desmethylimipramine was significantly greater than that at 5 μM ($F = 18.62$; d.f. 1,4; $P < 0.05$).

Effects of atipamezole alone, or in combination with medetomidine, on changes in noradrenaline efflux induced by medetomidine

Continuous perfusion of 0.5 μM atipamezole caused a progressive (time-dependent) increase in noradrenaline concentration in the dialysate which stabilized after 1 h ($F = 11.47$; d.f. 11,44; $P < 0.001$) (Figure 4). Overall, this increase was significant when compared with basal samples collected before drug administration ($F = 150.7$; d.f. 1,8; $P < 0.001$).

Noradrenaline efflux during continuous infusion of 0.5 μM atipamezole was compared with that in parallel samples collected in the presence of increasing concentrations of medetomidine alone (Figure 4). Although there was no main effect of drug ($F = 0.03$; d.f. 1,8; $P = 0.878$), there was a main effect of concentration ($F = 61.69$; d.f. 3,24; $P < 0.001$) and a drug × concentration interaction ($F = 17.29$; d.f. 3,24; $P < 0.001$). Noradrenaline efflux for each cluster of 3 samples at each concentration of medetomidine was therefore compared with that in time-matched samples collected during perfusion of 0.5 μM atipamezole. At 0.5 μM medetomidine, the main effect of drug (0.5 μM medetomidine *versus* 0.5 μM atipamezole) just failed to reach significance ($F = 4.84$; d.f. 1,8; $P = 0.059$). This is because the effects of atipamezole did not reach maximum until 1 h after the start of its perfusion. However, with this dose of medetomidine, there was a significant drug × time interaction ($F = 11.36$; d.f. 2,16; $P < 0.001$). Noradrenaline levels increased during perfusion of 5 μM medetomidine but they remained significantly lower than those in parallel samples taken during perfusion of

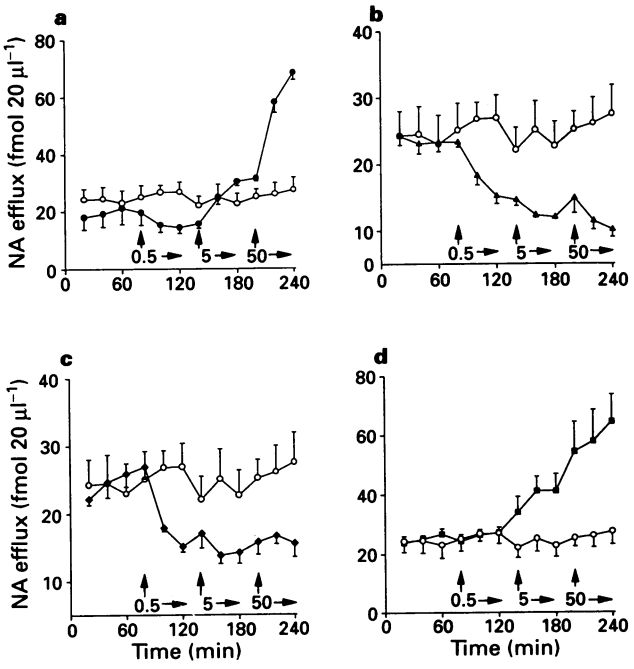

Figure 3 Effects of the imidazol(in)e α_2-adrenoceptor agonists (a) medetomidine (●), (b) clonidine (▲) and (c) UK 14,304 (◆) and the noradrenaline uptake inhibitor, (d) desmethylimipramine (■) on noradrenaline efflux in dialysates of the rat frontal cortex. Drugs were added to the perfusion medium in increasing concentrations (0.5 μM, 5.0 μM and 50 μM) and perfused for 60 min at the times indicated; (○) represents extended basal controls. Univariate ANOVA and multivariate statistical analyses are detailed in the Results section. Basal noradrenaline efflux (fmol 20 μl^{-1}; $n = 4$–5) was 21.2 ± 5.5 (●), 23.3 ± 1.4 (▲), 25.9 ± 2.0 (◆) and 26.6 ± 2.0 (■).

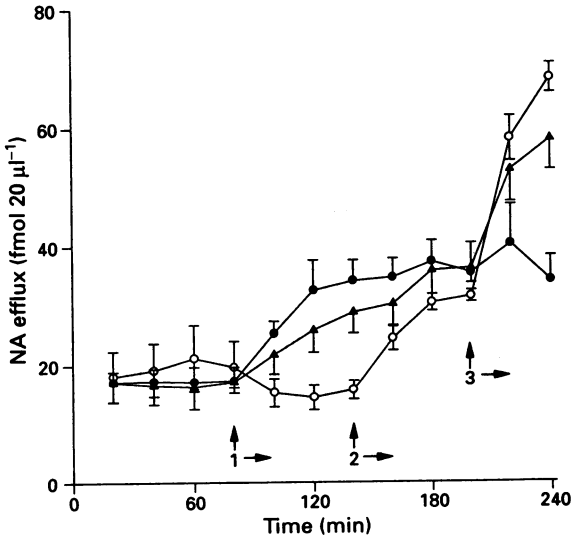

Figure 4 Effects of the selective α_2-adrenoceptor antagonist, atipamezole, on medetomidine-induced changes in cortical noradrenaline efflux (○). Atipamezole (0.5 μM) was continuously perfused from time (1), either alone (●), or combined with increasing concentrations of medetomidine (▲) as described in Figure 3 and indicated by arrows at (1) (0.5 μM), (2) (5.0 μM) and (3) (50 μM). Basal noradrenaline efflux was 17.2 ± 2.5 fmol 20 μl^{-1} ($n = 5$) (●), 16.3 ± 3.8 fmol 20 μl^{-1} ($n = 5$) (▲) and 21.2 ± 5.5 ($n = 5$) (○).

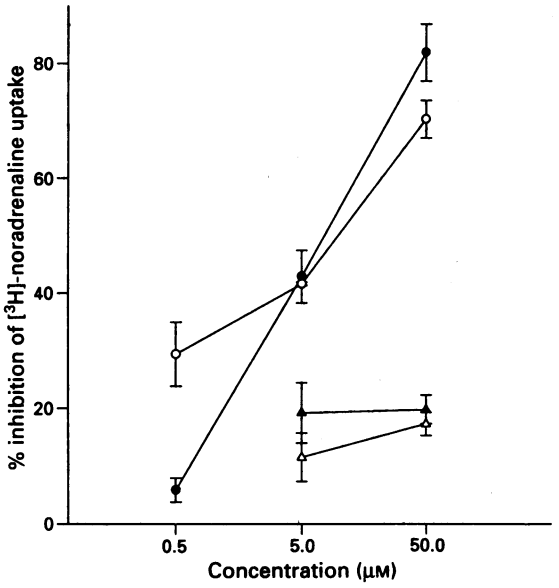

Figure 5 Concentration-dependent inhibition of [^{3}H]-noradrenaline uptake into cortical synaptosomes by medetomidine (●) and desmethylimipramine (○). Clonidine (▲) and UK 14,304 (△) were also tested. Each data point represents mean ± s.e.mean % inhibition of specific [^{3}H]-noradrenaline uptake ($n = 6-9$). Statistical analyses of the raw data are described in the text.

0.5 μM atipamezole alone ($F = 11.92$; d.f. 1,8; $P = 0.009$). In contrast, noradrenaline efflux during perfusion of 50 μM medetomidine was significantly greater than that in equivalent samples collected with atipamezole alone ($F = 13.64$; d.f. 1,8; $P = 0.006$).

Atipamezole (0.5 μM) was then co-administered with medetomidine (5–50 μM). When compared with medetomidine alone there was no main effect of drug ($F = 0.22$; d.f. 1,8; $P = 0.065$) over all samples, but there was a main effect of concentration ($F = 93.62$; d.f. 3,24; $P < 0.001$) and a significant drug × concentration interaction ($F = 3.39$; d.f. 3,24; $P = 0.034$) (Figure 4). Noradrenaline efflux was therefore compared between specific drug treatments. Comparison of noradrenaline efflux with 0.5 μM medetomidine and 0.5 μM medetomidine plus 0.5 μM atipamezole failed to expose a main effect of drug ($F = 2.21$; d.f. 1,8; $P = 0.175$). Multivariate tests also failed to show any drug treatment × time interaction ($P = 0.129$). Since comparison of medetomidine alone and atipamezole alone did expose a drug × time interaction (see above), it seems that changes in noradrenaline efflux induced by 0.5 μM medetomidine or 0.5 μM atipamezole are diminished when these drugs are given in combination. This suggests an additive interaction between these drugs at these concentrations. In contrast, there was no effect of atipamezole on noradrenaline efflux when this was co-administered with either 5.0 μM ($F = 4.44$; d.f. 1,8; $P = 0.068$) or 50 μM medetomidine ($F = 0.65$; d.f. 1,8; $P = 0.444$ *cf.* medetomidine alone).

Effects of medetomidine, UK 14,304, clonidine and desmethylimipramine on uptake of (−)-[^{3}H]-noradrenaline by cortical synaptosomes in vitro

Specific synaptosomal uptake of [^{3}H]-noradrenaline at 37°C was 1.02 ± 0.10 pmol mg^{-1} protein ($n = 9$). This uptake was inhibited by medetomidine with an IC_{50} of approximately 7 μM ($F = 11.15$; d.f. 3,35; $P < 0.001$) (Figure 5). Compared with the uptake in the absence of drug, *post hoc* tests exposed significant uptake inhibition at both 5 and 50 μM drug concentrations. Similar results were obtained with desmethylimipramine: this produced a significant, concentration-dependent reduction in synaptosomal uptake ($F = 6.52$; d.f. 3,42; $P = 0.01$) with *post hoc* tests exposing significant inhibition at both 5 and 50 μM concentrations. The IC_{50} for uptake inhibition was approximately 8 μM. In contrast, neither clonidine ($F = 0.11$; d.f. 2,11; $P = 0.896$) nor UK 14,304 ($F = 0.04$; d.f. 2,11; $P = 0.954$) produced any changes in synaptosomal uptake of [^{3}H]-noradrenaline.

Discussion

Systemic administration of medetomidine produces sedation, hypotension (Savola & Virtanen, 1991), antinociception (reviewed by Pertovaara, 1993), hypothermia (MacDonald *et al.*, 1988) and a reduction in general anaesthetic requirement (Bloor *et al.*, 1992). These effects have been attributed to activation of α_2-adrenoceptors since they are shared by other α_2-adrenoceptor agonists and are prevented by α_2-adrenoceptor antagonists. This is consistent with evidence from binding studies *in vitro* showing that medetomidine has a greater selectivity for α_2- *versus* α_1-adrenoceptors, and a higher affinity for binding to α_2-adrenoceptors than either clonidine or UK 14,304 (Virtanen *et al.*, 1988). In view of this binding profile, systemic administration of medetomidine should reduce noradrenaline release in the brain by activating both somatodendritic α_2-autoreceptors in the locus coeruleus and presynaptic α_2-adrenoceptors in the terminal field. Attenuated release of noradrenaline is suggested by a decline in brain levels of the noradrenaline metabolite, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) after systemic administration of a low dose of medetomidine (MacDonald *et al.*, 1991). Also, in the present study, a low micromolar concentration of medetomidine reduced noradrenaline efflux, whereas the α_2-adrenoceptor antagonist, atipamezole, increased it.

Despite this receptor selectivity, the main finding of the present study was that, at higher concentrations (5–50 μM), medetomidine markedly increased the levels of noradrenaline in cortical dialysates. Although unlikely to contribute to its clinical actions, this effect could be relevant to published reports that medetomidine has a biphasic effect on brain MHPG content: after an initial reduction, MHPG levels show a dose-dependent recovery (MacDonald *et al.*, 1991). It might also explain the reversal of sedation as the drug dose is increased beyond that recommended in anaesthesia (Doze *et al.*, 1989). It is also unlikely that the increase in noradrenaline efflux induced by medetomidine is related to its agonist action at α_2-adrenoceptors. This is because both clonidine and UK 14,304 were devoid of this effect, yet both these compounds are regarded as relatively potent and selective α_2-adrenoceptor agonists. More importantly, the increase in noradrenaline efflux was not prevented by co-administration of the α_2-adrenoceptor antagonist, atipamezole.

Attenuation of noradrenaline efflux by low, and increased efflux at high, drug concentrations could suggest that medetomidine has a lower efficacy at presynaptic α_2-adrenoceptors than noradrenaline. There are several reasons for discounting this possibility. First, the large increase in efflux caused by 50 μM medetomidine would indicate that α_2-adrenoceptors experience considerable tonic activation by spontaneously released noradrenaline. Yet, this is at variance with our results: the increase was no greater after treatment with atipamezole, either alone or in conjunction with medetomidine, than after medetomidine alone. Others have shown that, even high concentrations (100 μM) of selective α_2-antagonists, piperoxan and yohimbine, have little or no effect on noradrenaline levels in dialysates of the rat frontoparietal cortex (van Veldhuizen *et al.*, 1993). Interestingly, the imidazoline α_2-adrenoceptor antagonist, idazoxan

(100 μM), does cause an appreciable increase in efflux (150% with respect to basal: Dennis *et al.*, 1987), but this is considerably less than that reported here with medetomidine. Secondly, high concentrations of clonidine did not increase noradrenaline efflux despite evidence that this compound may act as a partial α_2-agonist (Medgett *et al.*, 1978). Finally, the presence of a large presynaptic α_2-adrenoceptor reserve in the cortex (Adler *et al.*, 1987) should lessen the impact of partial α_2-agonist activity on extraneuronal levels of noradrenaline.

An alternative explanation is that medetomidine inhibits uptake of noradrenaline from the extracellular fluid. To test this, the effects of medetomidine, clonidine and UK 14,304 on synaptosomal uptake of [^{3}H]-noradrenaline were compared with those of the uptake inhibitor, desmethylimipramine. Medetomidine inhibited [^{3}H]-noradrenaline uptake with a potency comparable with that of desmethylimipramine. This effect further distinguished medetomidine from clonidine and UK 14,304 which were both devoid of this activity. The high (micromolar) concentration of desmethylimipramine required to cause appreciable inhibition of [^{3}H]-noradrenaline uptake suggests that this mainly involved uptake into dopaminergic synaptosomes (Wood & Wyllie, 1983; Michel *et al.*, 1984). A similar action for medetomidine might therefore be inferred. Since both drugs inhibited synaptosomal uptake *in vitro* and increased efflux *in vivo* at the same concentrations, it is likely that these effects are related. This would indicate that much of the noradrenaline released in the cerebral cortex is sequestered by dopaminergic nerve terminals. This is entirely consistent with evidence that uptake of transmitter into noradrenergic neurones has a minor role in determining basal extracellular levels of noradrenaline in this brain area (Thomas & Holman, 1991).

An important finding of this study was that atipamezole did not modify the increase in noradrenaline efflux produced by medetomidine. In the presence of an uptake inhibitor, an α_2-autoreceptor antagonist like atipamezole should potentiate extracellular levels of noradrenaline through disinhibition of α_2-autoregulatory function. Presumably, these results reflect a competitive interaction between these two compounds at the α_2-autoreceptor.

To our knowledge, there are no previous reports of an α_2-adrenoceptor agonist inhibiting noradrenaline reuptake. Assuming structural differences to be important, it may be relevant that medetomidine is an imidazole derivative, whereas both clonidine and UK 14,304 are imidazolines. Whether this is the key distinguishing feature can only be resolved by structure-activity analyses, comparing the effects of a range of these compounds on noradrenaline efflux and synaptosomal uptake.

It is widely recognized that imidazol(in)es, as well as binding to α_2-adrenoceptors, share high affinity for non-adrenoceptor imidazoline/idazoxan binding sites (I-receptors: Wikberg *et al.*, 1991). These receptors, which seem to be anatomically, and possibly functionally, unrelated to α_2-adrenoceptors (Michel *et al.*, 1990; de Vos *et al.*, 1994), are selectively labelled by idazoxan in the presence of phenylethylamines or synthetic α_2-adrenoceptor ligands (Miralles *et al.*, 1993; Olmos *et al.*, 1993). Autoradiography studies have revealed a moderate density of I-receptors in the rabbit (Renouard *et al.*, 1993) and human (de Vos *et al.*, 1994) cerebral cortex. However, functional implications of I-receptor binding are poorly understood. Whilst there is no obvious link between the I-receptor binding affinities of medetomidine, clonidine and UK 14,304 in the rat brain (Miralles *et al.*, 1993) and their respective effects on synaptosomal noradrenaline uptake, it is evident that [^{3}H]-idazoxan binding in the cortex can be resolved into at least two components (Renouard *et al.*, 1993). Consequently, an I-receptor involvement in our results cannot be ruled out until agonist efficacies and full binding profiles to putative I-receptor subtypes have been characterized more fully.

One possible target linking transmitter uptake and I-receptor binding is the Na^+ -H^+ exchange pump of the neurolemma. I-receptors in the renal tubule, at least, modulate this proton pump (Bidet *et al.*, 1990) and changes in [Na^+] modify the affinity of the noradrenaline transporter protein for its substrate (Sammet & Graefe, 1979). This sequence of events could influence outward transport, as well as uptake, of noradrenaline (Langeloh & Trendelenburg, 1987). An extension of this mechanism involves the possible intracellular localisation of I-receptors (Tesson *et al.*, 1991) and their reported association with monamine oxidase (Olmos *et al.*, 1993; Renouard *et al.*, 1993). Modulation of monoamine oxidase activity by I-receptor ligands could indirectly affect neuronal uptake by altering axoplasmic concentrations of noradrenaline (Langeloh & Trendelenburg, 1987; Trendelenburg, 1989). This suggestion, that a receptor target for imidazol(in)es might be functionally coupled to the transmitter uptake transporter, is not new. An early scheme also proposed an imidazol(in)e site on the α_2-adrenoceptor which was coupled to the noradrenaline transporter (Gothert *et al.*, 1983; Starke, 1987) but results of recent investigations have not supported this theory (Zier *et al.*, 1988; Limberger *et al.*, 1990). Nevertheless, in the light of the present findings, the possibility that imidazol(in)e α_2-adrenoceptor ligands bind to a second, functionally distinct receptor which, albeit indirectly, influences noradrenaline uptake merits further consideration.

In summary, our results show that the selective α_2-adrenoceptor agonist, medetomidine, at micromolar concentrations, augments, rather than reduces, extraneuronal levels of noradrenaline in the rat frontal cortex. This effect appears to be largely due to inhibition of noradrenaline reuptake rather than any action at α_2-adrenoceptors. It remains to be determined whether medetomidine produces this effect *via* desmethylimipramine recognition sites on the uptake carrier complex.

We are indebted to Orion-Farmos, Boehringer Ingelheim and Pfizer for their generous gifts of drugs. We wish to thank Dr R. Virtanen for his helpful comments on the manuscript. This study was funded in part by the MRC (Great Britain).

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(Received October 6, 1994
Revised January 9, 1995
Accepted January 11, 1995)