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Potentiation of barbiturate-induced alterations in presynaptic noradrenergic function in rat frontal cortex by imidazol(in)e α_2-adrenoceptor agonists

[2,4]J.W. Dalley, [1]C.A. Parker, [3]E. Wülfert, [1]A.L. Hudson & [1]D.J. Nutt

[1]Psychopharmacology Unit, University of Bristol, Bristol, BS8 1TD; [2]Department of Experimental Psychology, Downing St, University of Cambridge, CB2 3EB and [3]UCB Pharma S.A., Chemin du Foriest, B-1420 Braine-l' Alleud, Brussels, Belgium

1 In order to resolve the extent to which presynaptic noradrenergic mechanisms contribute to the anaesthetic-sparing effects of α_2-adrenoceptor agonists *in vivo* microdialysis was used to investigate the combined effects of sodium pentobarbitone and imidazol(in)e α_2-adrenoceptor agonists on extracellular levels of noradrenaline (NA) in the rat frontal cortex.

2 Dialysate levels of NA were markedly reduced by the addition of TTX (2 μM) or by the removal of calcium in the perfusate. These data imply that dialysate NA levels are ultimately dependent on exocytotic release mechanisms from afferent coeruleo-cortical neurones.

3 Systemic administration of sodium pentobarbitone (8.5 mg kg^{-1}, i.p.) induced general anaesthesia and reduced NA levels by 92% after 30 min. The restoration of basal levels 90 min later was closely associated with a return of the corneal blink reflex.

4 Basal NA levels in conscious animals were not affected by an intravenous infusion of equally radioactive solutions of either imidazoline (clonidine) or imidazole (mivazerol) α_2-adrenoceptor agonists. The dose rate employed for each compound was 2 μg kg^{-1} h^{-1} over 2 h.

5 The co-administration of intravenous clonidine or mivazerol, each at 2 μg kg^{-1} h^{-1} for 2 h, with sodium pentobarbitone (8.5 mg kg^{-1}, i.p.), produced a marked and prolonged reduction in NA efflux. After 2 h, NA levels remained suppressed by 95% (clonidine) and 80% (mivazerol) and animals remained deeply anaesthetized.

6 The accumulation of tritium in brain tissue was 42–73% lower across all brain regions examined after [^{3}H]-mivazerol administration than after [^{3}H]-clonidine administration Sodium pentobarbitone did not alter the accumulation of tritium in brain tissue after the administration of either α_2-adrenoceptor agonist.

7 These data demonstrate that α_2-adrenoceptor agonists potentiate the inhibitory effects of sodium pentobarbitone on extracellular levels of NA in the frontal cortex. Further studies will be necessary to establish a causal role of noradrenergic mechanisms in the potentiation of anaesthesia by selective α_2-adrenoceptor agonists.

Keywords: mivazerol; clonidine; pentobarbitone; noradrenaline; GABA; anaesthesia

Introduction

Selective α_2-adrenoceptor agonists are widely reported to reduce the requirement of barbiturate (Mason & Angel, 1983; Scheinin *et al*., 1992) and other anaesthetic agents (Savola *et al*., 1991; Devcic *et al*., 1994). These anaesthetic-sparing actions are mediated centrally *via* the activation of α_2-adrenoceptors (Doze *et al*., 1989), however, no consensus has been reached regarding the relative involvement of pre- and postsynaptic α_2-adrenoceptors in this response. Somatodendritic and terminal α_2-adrenoceptors located on noradrenergic neurones have been widely implicated since halothane requirement is reduced by the destruction of central noradrenergic systems (Roizen *et al*., 1978; Segal *et al*., 1988). However, under these same conditions, the α_2-agonist, dexmedetomidine, is still able to reduce the minimum alveolar concentration of halothane in a dose-related, idazoxan-sensitive manner (Segal *et al*., 1988) implying that postsynaptic α_2-adrenoceptors may also be involved in this response.

In order to determine the extent to which presynaptic noradrenergic mechanisms contribute to the anaesthetic-sparing effects of α_2-adrenoceptor agonists the present study has used *in vivo* microdialysis to monitor extracellular levels of noradrenaline (NA) in the rat frontal cortex. An important objective of this study was to elucidate whether α_2-adrenoceptor agonists potentiate barbiturate-induced anaesthesia through convergent actions on NA release in terminal fields of the CNS. To test this hypothesis, clonidine and the more selective α_2-adrenoceptor agonist, mivazerol (Noyer *et al*., 1994), were tested in conscious and pentobarbitone-anaesthetized animals. Radioactive drugs were used in these studies to provide a measure of the relative distribution of α_2-adrenoceptor agonists in central and peripheral tissues following the administration of sodium pentobarbitone A preliminary account of these findings have been presented to the British Pharmacological Society (Dalley *et al*., 1996).

Methods

Animals

Requirements of the U.K. Animals (Scientific Procedures) Act, 1986 were followed throughout Male Wistar rats weighing between 240 g and 290 g were obtained from Bristol University (U.K.). Stock animals were housed in groups of

[4]Author for correspondence at present address: Department of Experimental Psychology, Downing Street, University of Cambridge, Cambridge CB2 3EB.

six to eight and allowed free access to food and water. Following surgery, animals were housed individually and allowed 24 h in which to recover.

Surgery

Concentric-design microdialysis probes were vertically implanted in the frontal cortex under sodium pentobarbitone anaesthesia (85 mg kg^{-1}, i.p.) according to the following stereotaxic co-ordinates (relative to bregma and the dural surface): anterior-posterior +3.0 mm; lateral ±3.2 mm; dorsoventral −4.0 mm (Paxinos & Watson, 1986). The dialysis probe was constructed using deactivated fused silica capillary tubing (140 μm o.d., SGE, U.K.) as the outlet line and 24 g thin wall stainless steel tubing (25 mm length) as the inlet line. The active portion of the probe consisted of a 3.5 mm length of Filtral 12 membrane (Hospal Industrie, France) which was sealed inside the inlet tubing using epoxy resin. The outlet line was fed through the wall of a 5 mm length of flexible solvent tubing (0.38 mm, i.d., 2.21 mm, o.d., Elkay Laboratory Products, U.K.) secured over the inlet tubing. The probe was secured to the skull surface using bone screws and dental cement. In those rats remaining conscious throughout the administration of saline, mivazerol or clonidine, a luer-lock catheter (Portex), primed with heparin (25 U ml^{-1}), was inserted into a femoral vein and externalised at the nape of the neck.

Microdialysis

Experiments commenced at the same time each day (10.00 am), approximately 24 h after probe implantation. The dialysis probe was perfused at a constant rate (2.0 μl min^{-1}) with artificial CSF of the following composition (mM); NaCl 146, KCl 3.0, $MgCl_2$ 1.0, $CaCl_2$ 1.2, sodium phosphate buffer 1.5, pH 7.4. The relative *in vitro* recovery of NA was approximately 16%. The dialysate collected over the first 60 min was discarded. Thereafter, dialysates were collected as 10 min (20 μl) fractions into 5 μl 0.01 M perchloric acid. Baseline levels of NA were determined over six to eight successive samples in the home-cage. In order to ensure that NA content in brain dialysates stemmed mainly from a neuronal origin, 2 μM tetrodotoxin (TTX) was added to the perfusate ($n=4$). In a further experiment, Ca^{2+} ions were removed from the perfusate for 80 min and then re-instated ($n=4$).

Following the determination of basal NA outflow, animals were injected with either 0.9% NaCl (2.0 ml kg^{-1}, i.p.) and maintained in their home-cage ($n=6$), or an anaesthetic dose of sodium pentobarbitone (85 mg kg^{-1}, i.p.) and placed on a heating blanket set at 37°C ($n=6$). In two animals, it was necessary to supplement the pentobarbitone dose by 10 mg kg^{-1}. Of these animals, one received intravenous clonidine while the other received no additional drugs. When animals were satisfactorily anaesthetized with pentobarbitone, a continuous intravenous infusion (2.4 ml h^{-1}) of either saline, clonidine or mivazerol (each group $n=6$) was commenced. This was achieved either *via* a tail vein for pentobarbitone-anaesthetized rats or *via* a preimplanted femoral vein cannula for animals remaining conscious throughout the experiment. The total drug dose of 2 μg kg^{-1} h^{-1} contained approximately 12% by weight of the respective tritiated compound (equivalent to 4.78 μCi ml^{-1}). This dose rate was chosen on the basis of a preliminary study showing that both drugs at this rate produce no discernible effects on spontaneous NA efflux in the conscious rat. After 2 h, the animals were sacrificed by anaesthetic overdose and cervical dislocation. Their brains were then removed and microdissected by region. Aliquots of brain, liver and kidney tissue were dissolved using soluene (20 μl mg^{-1}) and sonication. Tritium levels were determined by standard scintillation methods. The position of the cannula track in the cortex was confirmed by visual inspection.

Neurochemical analysis

NA levels in brain dialysates were determined by reversed-phase HPLC and electrochemical detection, essentially as described previously (Dalley & Stanford, 1995), but with some minor modifications. Briefly, the mobile phase (pH 5.0) contained sodium acetate (2.0 g L^{-1}), citric acid (4.0 g L^{-1}), EDTA (25 mg L^{-1}), sodium octane-sulphonic acid (400 mg L^{-1}) and methanol (10%) and was delivered at 1.0 ml min^{-1}. Separation was achieved using a Hypersil C18 ODS5 analytical column (4.6 mm i.d. × 10 cm length). Microdialysates and standards were manually injected *via* a Rheodyne 7125 valve (20 μl loop volume) and were oxidized at +300 mV (Coulochem I). The retention time of NA under these conditions was approximately 6 min and NA was clearly resolved from the solvent front. Peak height was used to quantitate NA levels in dialysates. The absolute detection limit of NA in aqueous standards was 1 fmole.

Drugs

Mivazerol hydrochloride and [^{3}H]-mivazerol hydrochloride were obtained from UCB S.A. Pharma (Belgium). Clonidine hydrochloride and [^{3}H]-clonidine hydrochloride were purchased from Fluka and Amersham International, respectively. Drug weights of mivazerol and clonidine were calculated in terms of the molecular weight of the free base. Sodium pentobarbitone ('Sagatal', 60 mg ml^{-1}, Rhone Merieux) was obtained locally.

Data analysis and statistics

The concentration of NA in brain dialysates is expressed as femtomoles per 20 μl of dialysate (mean ± s.e.mean) without correction for *in vitro* recovery. All statistical analyses were carried out using GB-STAT for Windows (Dynamic Microsystems Inc.). The data were analysed using repeated measures ANOVA with one between-subjects factor, TREATMENT (saline, clonidine or mivazerol) and one within-subject's factor, TIME (18 × 10 min bins). A *post-hoc* Newman-Keuls multiple comparisons test was used if the interaction between factors was significant ($P<0.05$). In this case pairwise mean comparisons were made between saline-treated and either clonidine-treated or mivazerol-treated animals at each time point. Comparisons were also made within each drug treatment group between the final basal sample (time = 0 min) and individual means. The data in Figure 1 were analysed using repeated measures ANOVA with TIME (24 × 10 min bins) as a within-subjects factor. Pairwise mean comparisons relative to the final basal sample (time = 0 min) were performed using a Dunnett multiple comparisons test. The central distribution of tritium was compared using 3-way ANOVA with factors, REGION (frontal cortex, hippocampus, cerebellum, caudate putaman, or spinal cord), ANAESTHETIC (conscious or anaesthetized) and DRUG (mivazerol or clonidine). A separate 3-way ANOVA was performed on tritium levels in kidney and liver tissue. *Post-hoc* tests were used where indicated by a significant main effect.

Results

The effects of calcium depletion and intraprobe TTX administration on basal extracellular levels of NA in the cortex are given in Figure 1. Basal levels of NA were significantly decreased by the removal of calcium from the perfusate. This effect reached statistical significance relative to the final basal sample (time = 0 min) 30 min after switching to a calcium-free perfusate with a maximum inhibition of 88% observed after approximately 90 min. NA levels were rapidly

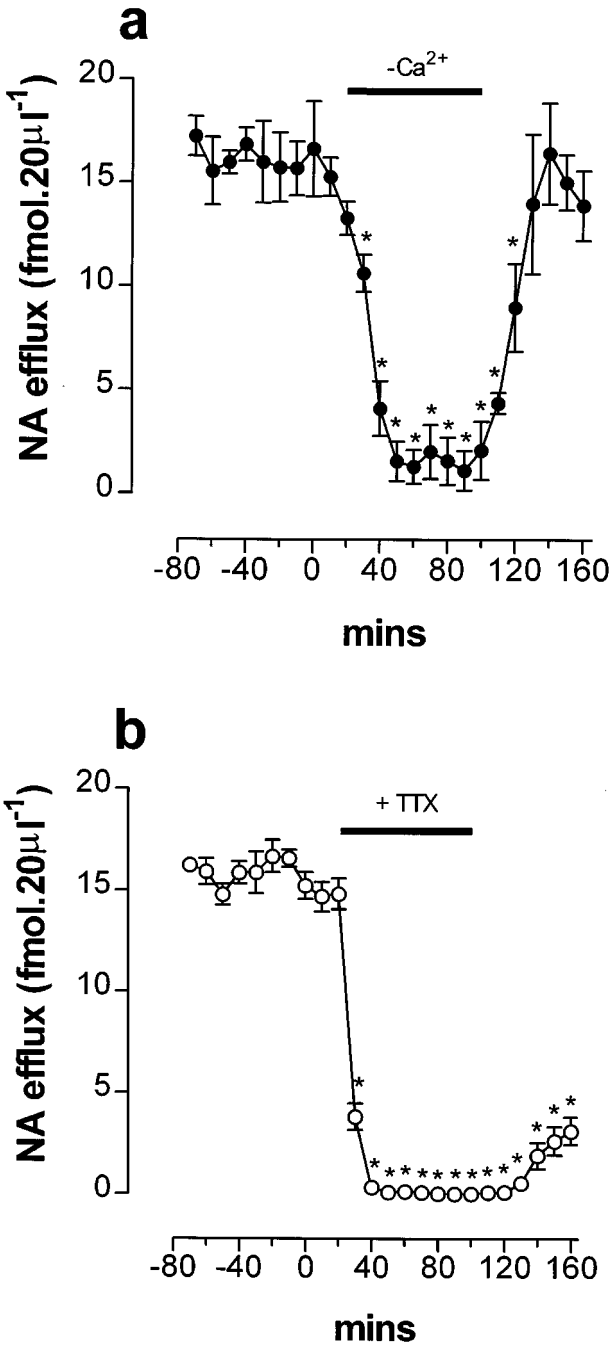

Figure 1 Effects of calcium depletion (a) and intraprobe TTX (2 μM) administration (b) on extracellular levels of NA in the frontal cortex of conscious unrestrained rats. The solid line indicates the time over which TTX and calcium were added or removed from the aCSF perfusate, respectively. The data are expressed as mean $\pm$ s.e.mean (each $n=4$). Basal NA levels (fmol 20 μl^{-1}) were 16.22 ± 1.19 (calcium) and 15.91 ± 0.42 (TTX). ANOVA revealed a significant main effect of time for both manipulations (calcium: $F_{23,95}=36.3$; $P<0.01$, TTX: $F_{23,95}=207$; $P<0.01$). $^*P<0.05$ denotes *post-hoc* within-group comparisons *versus* the final basal sample mean (time 0 min).

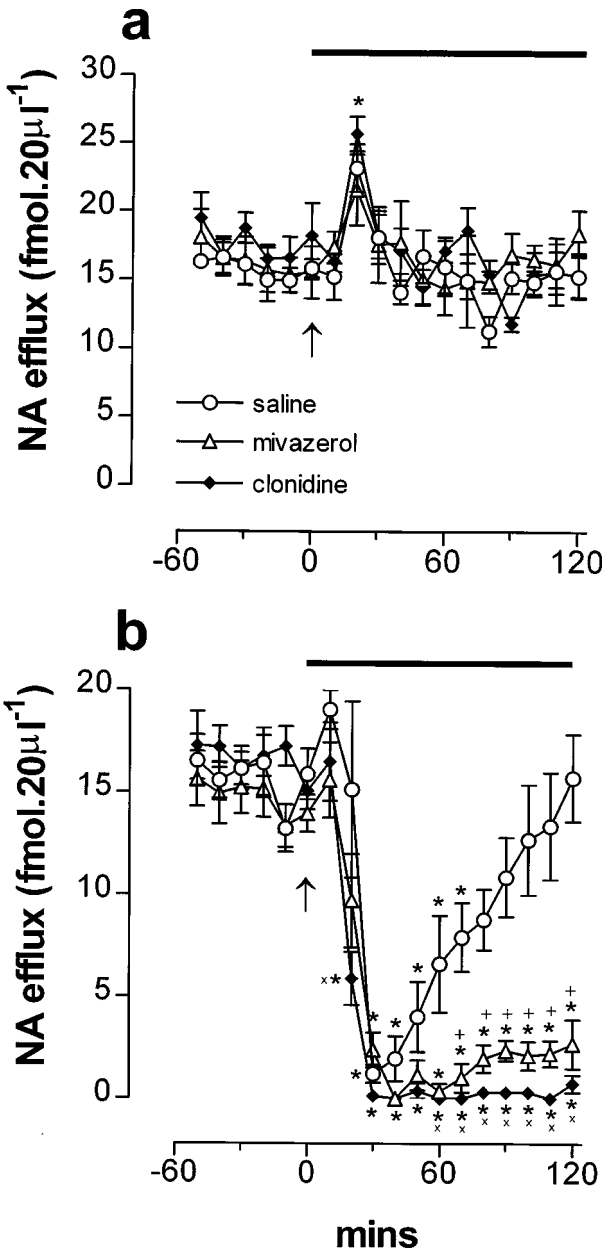

Figure 2 Effects of the α_2-adrenoceptor agonists, clonidine and mivazerol, administered either alone (a), or in combination with sodium pentobarbitone (b), on extracellular levels of NA in the rat frontal cortex. Clonidine and mivazerol were administered by continuous i.v. infusion (each at 2.0 $\mu g\ kg^{-1}\ h^{-1}$) at the time indicated by the solid line. Drug solutions were spiked with [^{3}H]-clonidine or [^{3}H]-mivazerol, respectively (final concentration 4.78 $\mu Ci\ ml^{-1}$). Controls animals received saline only (2.4 ml h^{-1}). Pentobarbitone (85 mg kg^{-1}, i.p.) or saline (2.0 ml kg^{-1}) were given by bolus i.p. injection at the times indicated by the arrow. The data are expressed as mean $\pm$ s.e.mean (each group $n=6$). Basal NA levels (fmol 20 μl^{-1}) in graph (a) were 15.82 ± 0.54 (saline), 17.72 ± 0.57 (clonidine) and 16.29 ± 1.06 (mivazerol). In graph (b), basal NA levels were 15.65 ± 0.46 (saline), 16.63 ± 0.54 (clonidine) and 14.73 ± 1.04 (mivazerol). $^*P<0.05$ denotes *post-hoc* within-group comparisons *versus* the final basal sample of each respective treatment group at time 0 min. $+P<0.05$ denotes *post-hoc* between-group comparisons between saline controls and mivazerol treated animals at each time point. $\times P<0.05$ denotes *post-hoc* comparisons between saline controls and clonidine-treated animals.

restored following the re-introduction of calcium in the perfusate. TTX (2 μM) produced a 95% reduction in basal NA efflux relative to the final basal sample (time = 0 min) with only modest recovery evident following its removal from the perfusate. These data demonstrate that physiological processes chiefly govern basal NA levels in accordance with previous findings (Dalley & Stanford, 1995). These findings help validate the assumption that such processes also operate during drug-evoked changes in NA efflux.

The effects of intravenously administered mivazerol and clonidine, either alone, or in combination with sodium pentobarbitone, on basal NA levels in the cortex are shown in Figure 2. When administered alone, neither mivazerol ($F_{1,10} = 0.46$; $P = 0.51$) nor clonidine ($F_{1,10} = 2.86$; $P = 0.12$) produced significant effects on extracellular levels of NA in the cortex relative to saline-treated control animals (Figure 2a). However, in both treatment groups, a significant main effect of time was obtained (mivazerol: $F_{17,215} = 2.85$; $P < 0.01$, clonidine: $F_{17,215} = 4.97$; $P < 0.01$), which, in the case of clonidine, reached statistical significance at the 20 min time point relative to the final basal sample (time = 0 min). This corresponded with the connection of the infusion line to the i.v. catheter. No treatment × time interactions were detected in this experiment.

When administered alone, sodium pentobarbitone produced anaesthesia as judged by the complete absence of the corneal blink reflex to non-noxious tactile stimuli and significantly reduced NA efflux (Figure 2b). The maximum inhibition of NA efflux of approximately 92% was observed after 30 min. Thereafter, NA levels remained significantly suppressed for a further 40 min relative to the final basal sample (time = 0 min). By 120 min, animals were clearly sedated but all showed a return of somatosensory reflex function.

NA levels showed a marked decline over the entire treatment time course when sodium pentobarbitone was co-administered with either mivazerol or clonidine (Figure 2b). 2-way ANOVA revealed a significant main effect of drug treatment on NA levels relative to pentobarbitone-anaesthetized animals (mivazerol: $F_{1,10} = 18.57$; $P < 0.01$, clonidine: $F_{1,10} = 22.41$; $P < 0.01$), a significant main effect of time (mivazerol: $F_{17,215} = 31.2$; $P < 0.01$, clonidine: $F_{17,215} = 43.7$; $P < 0.01$) and a significant treatment by time interaction (mivazerol: $F_{17,215} = 4.41$; $P < 0.01$, clonidine: $F_{17,215} = 9.15$; $P < 0.01$). *Post-hoc* tests revealed that NA levels were significantly reduced compared to NA levels in pentobarbitone-anaesthetized animals 70 min after the start of the mivazerol infusion and after 60 min in the case of clonidine-treated animals. The dissociation in NA efflux persisted in both treatment groups for the duration of the experiment. Further comparisons revealed that the co-administration of clonidine with pentobarbitone produced a more rapid decline in NA levels relative to saline controls during the first 20 min of administration compared with that seen in mivazerol-treated animals. In contrast to rats anaesthetized with sodium pentobarbitone only, clonidine and mivazerol-treated animals remained deeply anaesthetized at the end of the experiment (120 min) as judged by the complete absence of the corneal blink reflex.

The accumulation of tritium in central and peripheral tissues following the intravenous administration of [^{3}H]-clonidine or [^{3}H]-mivazerol in conscious and pentobarbitone-anaesthetized rats is shown in Table 1. ANOVA revealed a significant main effect of drug treatment ($F_{1,99} = 72.44$; $P < 0.01$), no effect of anaesthesia ($F_{1,99} = 1.56$; $P = 0.21$) and no effect of brain region ($F_{4,99} = 1.63$; $P = 0.17$). *Post-hoc* analysis revealed that the accumulation of tritium in conscious subjects following mivazerol administration was significantly lower across all brain regions compared with tritium accumulation following clonidine administration. In pentobarbitone-anaesthetized animals, the accumulation of tritium was significantly lower in cortical tissue versus [^{3}H]-clonidine-treated animals. Similar analysis of the peripheral data revealed significant main effects of anaesthesia ($F_{1,38} = 7.82$; $P < 0.01$) and region ($F_{1,38} = 7.76$; $P < 0.01$) and significant interactions between anaesthesia and drug treatment ($F_{1,38} = 4.17$; $P = 0.048$) and between region and drug treatment ($F_{1,38} = 15.99$; $P < 0.01$). *Post hoc* tests revealed that tritium accumulation was greatest in liver and kidney tissue following the administration of [^{3}H]-mivazerol and [^{3}H]-clonidine in conscious animals, respectively. These levels were significantly attenuated by prior sodium pentobarbitone administration.

Discussion

Selective α_2-adrenoceptor agonists are widely reported to reduce the requirement of volatile (Devcic *et al*., 1994; Savola *et al*., 1991), opiate (Ghignone *et al*., 1986) and barbiturate (Mason & Angel, 1983; Scheinin *et al*., 1992) anaesthetic agents. Whilst central α_2-adrenoceptors have been clearly implicated in this effect (Doze *et al*., 1989) few details have emerged regarding their synaptic location and functional coupling. A case has been made for a role of presynaptic α_2-adrenoceptors on the grounds that generalised NA depletion in the CNS reduces halothane demand in the rat (Roizen *et al*., 1978). However, this view has been challenged by the report that the selective α_2-agonist, dexmedetomidine, still reduces anaesthetic demand even after the selective depletion of brain NA content (Segal *et al*., 1988).

The primary objective of this study was to establish whether α_2-agonists potentiate barbiturate-induced anaesthesia through

Table 1 Tritium concentration in *post-mortem* tissue following i.v. [^{3}H]-clonidine and [^{3}H]-mivazerol administration (each 4.78 μCi ml^{-1}) over 2 h in sodium pentobarbitone and saline-treated rats

	Saline		*Sodium pentobarbitone*	
	Mivazerol	*Clonidine*	*Mivazerol*	*Clonidine*
Frontal cortex	28.5 ± 2.0*	103.2 ± 9.1	27.9 ± 6.7*	86.7 ± 26.1
Hippocampus	30.8 ± 2.2*	87.5 ± 5.8	44.0 ± 10	85.8 ± 28.9
Cerebellum	26.5 ± 1.6*	69.6 ± 5.1	30.0 ± 8.5	59.6 ± 19.5
Caudate putamen	31.3 ± 2.9*	88.3 ± 4.3	25.4 ± 8.6	56.3 ± 19.6
Spinal cord	29.3 ± 2.4*	73.0 ± 4.8	22.6 ± 6.0	61.8 ± 15.8
Liver	763 ± 26*	174 ± 17	280 ± 88†	101.1 ± 17.2
Kidney	246 ± 39*	955 ± 164	619 ± 186†	317.7 ± 100†

The data are expressed as mean d.p.m. mg^{-1} tissue ± s.e.mean ($n = 6$). *$P < 0.05$ mivazerol *versus* clonidine with respect to either saline or pentobarbitone treatment groups. †$P < 0.05$ saline *versus* pentobarbitone with respect to either mivazerol or clonidine.

convergent actions on NA release in the frontal cortex. The results clearly demonstrate that α_2-agonists act to potentiate the inhibitory effects of pentobarbitone on NA efflux in this terminal field. Unfortunately, given the correlational nature of the data, no inference can be made linking a causative role of reduced NA release to the potentiation of barbiturate-induced anaesthesia by α_2-agonists. For example, possible dissociable effects of α_2-adrenoceptor agonists on pre- and postsynaptic α_2-adrenoceptors affecting NA release and depth of anaesthesia, respectively, cannot be ruled out by these data. Furthermore, since the *in vivo* receptor specificity of clonidine and mivazerol for α_2-adrenoceptors at 2.0 μg kg h^{-1} is unproven their precise mechanism of action is unknown.

The finding that pentobarbitone reduces cortical NA efflux is in broad agreement with other studies. Thus, barbiturates not only reduce brain NA turnover (Persson & Waldeck, 1971; Lidbrink *et al*., 1972) they also attenuate K^+-evoked [^{3}H]-NA overflow from mouse forebrain synaptosomes (Haycock *et al*., 1977) and rat cortical slices (De Boer *et al*., 1982; Jones & Symington, 1990). Indeed, a close relationship exists between the IC_{50} of pentobarbitone to inhibit K^+-evoked [^{3}H]-NA overflow from cortical slices of approximately 100 μM (De Boer *et al*., 1982; Jones & Symington, 1990) and its *in vivo* EC_{50} of 50 μM to prevent movement to painful stimuli (Franks & Lieb, 1994). However, in a recent *in vivo* microdialysis study no change in basal NA efflux was reported in the rat prefrontal cortex following a 50 mg kg^{-1} i.p. administration of pentobarbitone (Pan & Lai, 1995). Although this finding appears to rule out a causative role of NA in barbiturate anaesthesia it does not rule out a role of this transmitter in the anaesthetic potentiating effects of selective α_2-agonists.

$GABA_A$ receptors, particularly those of the $\alpha_3\beta\gamma_2$ subtype present on cholinergic and monoaminergic neurones in cortical and other terminal areas, are thought to regulate amine transmitter turnover (McKernan & Whiting, 1996). Whilst the events which follow the allosteric interaction of pentobarbitone with $GABA_A$ receptors to elicit reduced NA release in the cortex are unclear there are reasonable grounds for considering presynaptic inhibition (Brown *et al*., 1982). Thus, barbiturates not only block the uptake of ^{45}Ca into depolarized nerve terminals (Blaustein & Ector, 1975) they also inhibit spontaneous and K^+-evoked changes in intracellular Ca^{2+} concentration (Bleakmen *et al*., 1995). Surprisingly, however, most voltage-gated ion channels including calcium channels appear to be relatively insensitive to inhibition by barbiturates and other anaesthetic agents at clinically relevant concentrations (Franks & Lieb, 1994). Nevertheless, in addition to GABA, NA also modulates presynaptic calcium channel function (Brown *et al*., 1982) suggesting that interactive effects of GABA and NA on calcium entry and function in noradrenergic terminals may underlie our observations.

The main finding of this study was that α_2-agonists potentiate the inhibitory effects of pentobarbitone on NA efflux in the frontal cortex. The finding that mivazerol can still elicit this response despite poor CNS entry suggests that this effect is remarkably potent. Since tritium levels in *post mortem* brain tissue (an approximation to drug levels) were unaltered by pentobarbitone this interaction is likely to be receptor based and not pharmacokinetic. The high affinity of [^{3}H]-mivazerol for hepatic tissue and its displacement by pentobarbitone suggests that in common with other imidazoles (Rogerson *et al*., 1977) mivazerol binds to microsomal cytochrome P-450. Therefore, mivazerol may exert its anaesthetic potentiating effects by reducing pentobarbitone metabolism. However, a more general case for a centrally mediated interaction between α_2-agonists and pentobarbitone can be argued on the grounds that clonidine does not inhibit microsomal cytochrome P-450 function (Kharasch *et al*., 1991) and excessive sedation precludes the determination of the inhibitory effects of the imidazole α_2-agonist, dexmedetomidine on cytochrome P-450 metabolism (Pelkonen *et al*., 1991). Possible mechanisms underlying this interaction may relate to presynaptic inhibition, enhanced GABA release in response to α_2-agonist administration (Pittaluga & Raiteri, 1988; Maura *et al*., 1988) and the recognised functional link between adenylate cyclase and $GABA_A$ receptors (Kirkness *et al*., 1989; Browning *et al*., 1990; Porter *et al*., 1990; Leidenheimer *et al*., 1991).

In summary, this study provides direct evidence that α_2-agonists and barbiturates act in a concerted manner to inhibit extracellular levels of NA in the frontal cortex. Whilst NA levels appear to be closely related to the potentiation of pentobarbitone-induced anaesthesia the data do not rule out confounding effects of postsynaptic α_2-adrenoceptors in this response. Further studies will be necessary to determine the significance of this interaction to the potentiation of general anaesthesia by selective α_2-adrenoceptor agonists.

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