2). Since some of these compounds had lipophilic properties similar to those of 1,3,8-TSX, as can be deduced from their reverse phase chromatographic retention times (Tables 2 and Christensen & Whitsett 1976), the accumulation of caffeine by the 1,3,8-TSX appears to be a function of their trisubstitution rather than of their overall lipophilic characteristics.

In conclusion, and based upon previously experimental observation (Tarrús et al 1987a,b), the results presented in this work indicate that compounds with a methyl group in position C^8, in addition to both N^1-methyl and N^3-aryl or alkyl substitution of the xanthine nucleus, are able to produce an accumulation of concurrently administered caffeine in blood, probably due to an inhibition of its metabolism. Our results also suggest that 1,3-disubstituted xanthines do not affect the metabolism of concurrently administered caffeine.

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Interaction between the cardiovascular effects of clonidine and the κ-opioid agonist U-50,488H in the anterior hypothalamic area of the rat brain

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Abstract—In thiobutabarbitone-anaesthetized rats, microinjection of clonidine (1–40 nmol) into the anterior hypothalamic area (AHy) produced dose-dependent reductions in mean arterial blood pressure and heart rate. Microinjection of the κ-opioid agonist U-50,488H (3 and 10 nmol) did not modify these parameters. Simultaneous co-administration of clonidine (4 nmol) and U-50,488H (10 nmol) into the AHy resulted in significant potentiation of the clonidine-induced hypotension and marked attenuation of the bradycardia. A lower dose of U-50,488H (3 nmol) co-administered with clonidine (4 nmol) did not influence the cardiovascular responses to clonidine. These findings suggest that AHy neurons involved in the cardiovascular responses to clonidine may be modulated by κ-opioid receptor stimulation.

The anterior hypothalamic area (AHy) of the rat brain is a site of central cardiovascular regulation (see Brody et al 1980; Abboud 1984) functioning as an integrative centre (Ciriello et al 1983) and as a modulatory influence on the baroreceptor reflex (Miyajima & Bunag 1985). Adrenoceptors of the α_2-subtype are present in the AHy (Young & Kuhar 1980; Unnerstall et al 1984) and their stimulation by direct microinjection of the imidazolidine derivative clonidine results in hypotension and bradycardia (Struyker-Boudier et al 1974). Similarly, opioid receptors of the κ subtype have been detected in the AHy (Lynch et al 1985) whilst the presence of the putative endogenous κ receptor agonist dynorphin (Corbett et al 1982) has been demonstrated immunohistochemically in this brain region (Khachaturian et al 1982). However, intrahypothalamic injection of κ-agonists in conscious rats produced no detectable cardiovascular effects (Pfeiffer et al 1982). On the other hand, McWilliam & Campbell (1987) have demonstrated that α_2-adrenoceptors and κ opioid receptors may interact in rat hypothalamic synaptosomes to modulate the release of noradrenaline.

In the present study we have examined the cardiovascular effects of co-administration into the AHy of the selective κ agonist U-50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl] benzeocetamide) (Vonvoigtlander et al 1983) and clonidine in anaesthetized rats.

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Materials and methods

Male Wistar-Kyoto rats weighing between 250–350 g were anaesthetized with thiobutabarbitone sodium (Inactin, 100 mg kg^{-1}, intraperitoneally). The rats were tracheotomized and a polyethylene catheter (SP 45) was inserted into the right carotid artery for blood pressure and heart rate (HR) measurements. The arterial catheter was connected to a Gould-Statham pressure transducer which was coupled to a Grass polygraph recorder. HR was derived from the pressure signal using a tachometer (Grass, model 7P44B). The rats were then placed into a stereotaxic apparatus (David Kopf) and stainless steel needles (300 μm O.D.) were lowered bilaterally through cranial burr holes into the AHy. Stereotaxic co-ordinates were: A.P. −1·1 to −1·6 mm from bregma; ±0·5 mm lateral to midline; 9·0 mm ventral to the skull surface, in the flat skull position (Paxinos

& Watson 1982). Not less than 5 min later, the rats received bilateral microinjections (0·5 μL) of normal saline (0·9% NaCl w/v) or artificial cerebrospinal fluid (ACSF), clonidine HCl (1, 4, 10 and 40 nmol), U-50,488H (3, 10 or 100 nmol) or clonidine HCl (4 nmol) co-administered with U-50,488H (3 or 10 nmol). Microinjections were made over 1·8 min and only one injection was performed in each rat. The composition of the ACSF was (mM): NaCl 127; KCl 3·0; $NaH_2PO_4 2H_2O$ 0·48; $NaHCO_3$ 2·6; $CaCl_2$ 1·8; $MgSO_4 7H_2O$ 0·4; glucose 3·6; urea 3·3. Blood pressure and HR were recorded for 40 min following the start of the injections. Body temperature was maintained throughout the experiment with a heating blanket. At the end of the experiment each rat was transcardially perfused with phosphate-buffered formalin (pH 7·4) and the location of the cannula tips in the AHy was verified histologically in Pyronine Y stained sections (40 μm). Only data from experiments in which the cannula tips were within the AHy were included in the analysis. Cardiovascular data were expressed as mean ± s.e.m. and analysed by analysis of variance with repeated measures BMDP statistical package (Department of Mathematics, University of Los Angeles, Los Angeles, USA) followed by Student's modified *t*-test using the Bonferroni modification for between group comparisons (Wallenstein et al 1980).

Results

Injection of saline or ACSF (0·5 μL) did not significantly modify mean arterial blood pressure (MAP) or HR ($P > 0.05$ for both comparisons (Fig. 1). Resting MAP and HR values in this group were 132 ± 5 mmHg and 359 ± 17 beats min^{-1} (n = 5 rats), respectively. These values did not differ significantly from those

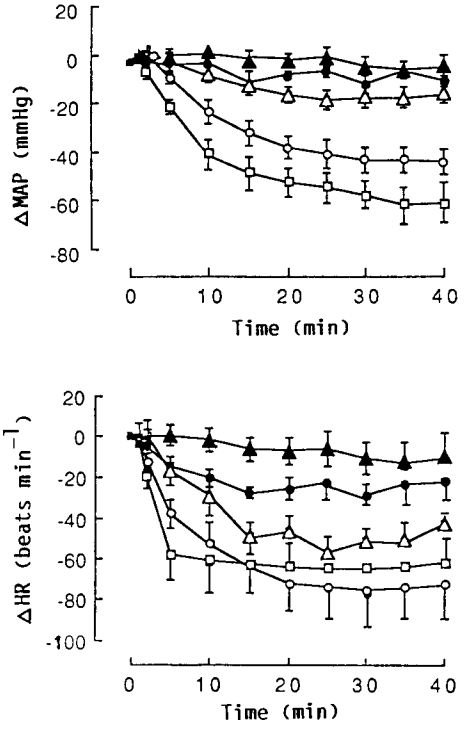

FIG. 1. Changes in mean arterial pressure (MAP, upper panel) and heart rate (HR; lower panel) produced by clonidine HCl (1 nmol, ●——●; 4 nmol, △——△; 10 nmol, ○——○; 40 nmol, □——□) and saline (▲——▲) after microinjection into the anterior hypothalamic area of anaesthetized rats. Results are mean ± s.e.m. (n = 4–6 rats/group). Microinjections were made at time t = 0 min. Some standard error bars have been omitted for the sake of clarity.

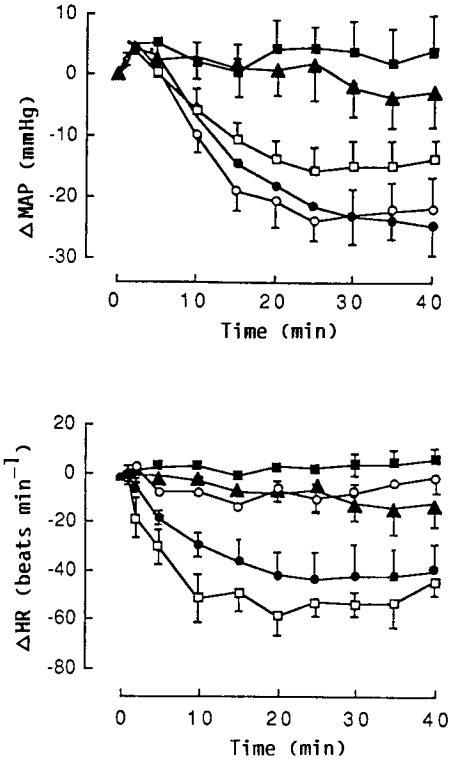

FIG. 2. Changes in mean arterial pressure (MAP, upper panel) and heart rate (HR, lower panel) produced by saline (▲——▲), clonidine HCL (4 nmol, □——□), U-50,488H (10 nmol, ■——■) and clonidine HCL (4 nmol) co-administered with U-50,488H (3 nmol, ●——●; 10 nmol, ○——○) after microinjection into the anterior hypothalamic area of anaesthetized rats, results are mean ± s.e.m. (n = 4–5 rats/group). Microinjections were made at time t = 0 min. The curve for U-50,488H (3 nmol) has been omitted for the sake of clarity but was essentially similar to that of U-50,488H (10 nmol). Some standard error bars have been omitted for the sake of clarity.

observed in the other treatment groups. In contrast, clonidine (4–40 nmol) produced dose-dependent hypotensive responses which developed over 10–15 min and lasted for the duration of the observation period ($P < 0.05$). The lowest dose of clonidine tested (1 nmol) did not produce a significant hypotensive response ($P > 0.05$). The bradycardic effects of clonidine (1–40 nmol) were also dose-dependent with the maximal effect occurring with the 10 nmol dose. Microinjection of U-50,488H (10 nmol) into the AHy did not produce any significant cardiovascular response compared to saline ($P > 0.05$) (Fig. 2), whereas microinjection of clonidine (4 nmol) resulted in both hypotension and bradycardia. Lower (3 nmol) and higher (100 nmol) doses of U-50,488H also failed to affect MAP or HR (data not shown). Co-administration of clonidine (4 nmol) with U-50,488H (10 nmol) resulted in marked attenuation of the bradycardic response to clonidine and a modest potentiation of the hypotension ($P < 0.05$ for both comparisons). Co-administration of a smaller dose of U-50,488H (3 nmol) with clonidine (4 nmol) did not alter MAP or HR responses to clonidine ($P > 0.05$ for both comparisons).

Discussion

The present study has demonstrated that activation of κ receptors within the AHy may interact with α_2-adrenoceptor stimulation. This effect appears to be restricted to κ receptors since in pilot experiments morphine (10 nmol) was found to be ineffective (Verberne, unpublished observations). Furthermore,

these observations confirm and extend the findings of Struyker-Boudier et al (1974) in providing evidence for a hypothalamic site of action for clonidine. However, it is apparent from other studies that clonidine may have multiple sites of action for producing cardiovascular effects including sites within the ventrolateral medulla (Bousquet et al 1984; Punnen et al 1987). Co-administration of clonidine with U-50,488H resulted in marked attenuation of the bradycardic response to clonidine accompanied by a slight potentiation of the hypotensive response. These divergent actions may result from the possibility that the bradycardic and hypotensive actions of clonidine elicited from the AHy are not necessarily mediated by identical neuronal elements. Electrophysiological studies have demonstrated that κ receptor stimulation leads to inhibition of neuronal firing in the brainstem and caudate nucleus (Bradley & Brookes 1984), as well as in the hippocampus (Bradley & Brookes 1984; Moises & Walker 1985). Therefore, it is conceivable that activation of κ receptors in the AHy may modulate the excitability of the neuronal pathway(s) involved in the cardiovascular responses to clonidine. Whether interactions between α_2-adrenoceptors and κ receptors occur at sites other than the AHy is at present unknown. An interaction between α_2-adrenoceptors and κ receptors has been observed in rabbit brain cortex (Limberger et al 1986) while the endogenous κ receptor agonist dynorphin (1–13) (Chavkin et al 1982) has been shown to reduce the inhibitory effects of clonidine on [^{3}H]noradrenaline release from rat hypothalamic synaptosomes (McWilliam & Campbell 1987). Conceivably these observations and the present findings may be manifestations of a general phenomenon where α_2-adrenoceptors and kappa receptors interact to modulate the actions of certain neurones. Until the neuroanatomical relationship between α_2-adrenoceptors and κ receptors is understood the neuropharmacological significance of these interactions will remain unclear.

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