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Evidence that locus coeruleus is the site where clonidine and drugs acting at α_1- and α_2-adrenoceptors affect sleep and arousal mechanisms

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1 The behavioural and electrocortical (ECoG) effects of clonidine were studied after microinjection into the third cerebral ventricle, or microinfusion into some specific areas of the rat brain rich in noradrenaline-containing cell bodies (locus coeruleus) or into areas receiving noradrenergic terminals (dorsal hippocampus, amygdaloid complex, thalamus, frontal and sensimotor cortex).

2 The ECoG effects were continuously analysed and quantified by means of a Berg-Fourier analyser as total power and as power in preselected bands of frequency.

3 Clonidine (9.4 to 75 nmol) given into the third cerebral ventricle produced behavioural sedation and sleep and a dose-dependent increase in ECoG total voltage power as well as in the lower frequency bands. Much lower doses were required to produce similar behavioural and ECoG spectrum power effects after either unilateral or bilateral microinfusion of clonidine into the locus coeruleus.

4 Doses of clonidine equimolar to those given into the third cerebral ventricle, were almost ineffective in inducing behavioural and ECoG sleep after their microinfusion into the dorsal hippocampus. In addition, a dose (0.56 nmol) of clonidine which, given into the locus coeruleus, produced marked behavioural sleep and ECoG synchronization, lacked effects when given into the ventral or anterior thalamus, into the amygdaloid complex or onto the frontal and sensimotor cortex.

5 The behavioural and ECoG spectrum power effects of clonidine given into the third cerebral ventricle or into the locus coeruleus were prevented by antagonists of α_2-adrenoceptors but not by α_1-adrenoceptor antagonists.

6 Intraventricular microinjection, or microinfusion into the locus coeruleus, of yohimbine, a selective α_2-adrenoceptor antagonist, produced behavioural arousal, increase in locomotor and exploratory activity, tachypnoea and ECoG desynchronization with a significant reduction in total voltage power. Similar stimulatory effects were also observed after microinjection of phentolamine into the same sites.

7 No significant effects on behaviour and ECoG activity were evoked after intraventricular injection or microinfusion into the locus coeruleus of prazosin or methoxamine.

Introduction

One of the most common side-effects of clonidine and clonidine-like compounds is sedation, which, like the hypotensive and hypothermic effects of these compounds is mediated by activation of central α-adrenoceptors (Brünner & Klein, 1968; Marley & Nisticò, 1975; Van Zwieten, 1975; Drew *et al.*, 1979). Evidence has accumulated during the last decade on the existence of two subtypes of α-adrenoceptors, termed α_1 and α_2 (see Langer, 1974; Starke & Langer, 1979; Starke, 1980). Most of the central actions of clonidine result from the stimulation of α_2-adrenoceptors (Cavero & Roach, 1978; Drew *et al.*, 1979; Depoortere, 1981; Timmermans *et al.*, 1981; Rotiroti *et al.*, 1983).

As far as the hypotensive and bradycardic effects of clonidine are concerned there is evidence that these are mediated through the nucleus of the tractus solitarius, the nucleus ambiguous of the medulla and the hypothalamic vasomotor centres (Schmitt *et al.*, 1971; Philippu & Schartner, 1976; De Jong & Nijkamp, 1976; Sinha *et al.*, 1985).

However, little is known about the neuroanatomical

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sites at which clonidine and related compounds exert their sedative effects. Therefore, we attempted to ascertain the sites through which behavioural and electrocortical spectrum power effects of clonidine and clonidine-like drugs were mediated. It is well known that the locus coeruleus represents an important area from which the main ascending and descending noradrenergic pathways in the brain originate (Dahlström & Fuxe, 1964). In particular, a dorsal ascending pathway projects its terminals diffusely into the cerebral cortex, the hippocampus and the cerebellar cortex, whereas a ventral ascending pathway innervates brain-stem structures, the hypothalamus, dorsal thalamus, amygdaloid nuclei and other subcortical areas (Ungersted, 1971; Morrison *et al.*, 1978; Moore & Card, 1984). In addition, it has been recognized for a long time that the locus coeruleus is a crucial area in the control of the sleep-waking cycle; in particular, noradrenaline containing neurones present in the locus coeruleus have been suggested to be involved in initiating or maintaining stages of the sleep-waking cycle (Steriade & Hobson, 1976; Amaral & Sinnamon, 1977; Ramm, 1979; Clark, 1979). Electrophysiological data supporting this view have been obtained for cats (Chu & Bloom, 1974; Hobson *et al.*, 1975; Sakai, 1980) and rats (Aston-Jones & Bloom, 1981a).

Thus we have compared the behavioural and electrocortical spectrum power effects of clonidine after its infusion in areas in which noradrenaline containing perykaria are located, i.e. locus coeruleus, as well as in several areas in which there are diffuse projections of noradrenergic nerve endings, i.e. frontal and sensimotor cortex, dorsal hippocampus, amygdaloid complex, anterior and ventral nuclei of the thalamus. In addition, we determined, by the use of specific antagonists of α_1- or α_2-adrenoceptors, whether the sedative effects of clonidine were mediated through activation of a specific subtype of adrenoceptor and whether noradrenergic pathways originating from the locus coeruleus exert a tonic influence on the projection areas.

A preliminary account of these findings has been presented at a Joint Meeting of the British and Italian Pharmacological Societies (Ascioti *et al.*, 1985).

Methods

Adult male Wistar rats (200–250 g) were purchased from Morini (San Polo D'Enza, Reggio Emilia) and maintained on a 12 h light-dark cycle (lights on 6 h 00 min–18 h 00 min, off 18 h 00 min–6 h 00 min). Animals were stereotaxically implanted with stainless steel guide cannulae under ketamine hydrochloride anaesthesia, according to the atlas co-ordinates of Paxinos & Watson (1982), to permit drug injection into third cerebral ventricle, dorsal hippocampus, amygdaloid complex, thalamus (ventral and anterior nuclei) and frontal and sensimotor cortex. In addition, in other rats a unilateral or bilateral steel guide cannula was implanted at an angle of 5° from the vertical, with the tip 2 mm away from the locus coeruleus. After surgery a minimum of 48 h was allowed for recovery before experiments were carried out. All experiments were performed beginning at approximately 10 h 00 min. Freely moving rats were microinjected (0.2 μl min^{-1}) via the injector cannula which extended approx. 2 mm below the tip of the guide cannula. Histological examination post-mortem confirmed the location of the guide cannulae.

Electrocortical (ECoG) activity was recorded (8 channel ECoG machine OTE BIOMEDICA, Florence) via 4 chronically implanted steel screw electrodes inserted onto each fronto-parietal area. For statistical purposes, the bipolar signals from each fronto-parietal area were integrated by means of a Berg-Fourier analyser (OTE BIOMEDICA) according to Bricolo *et al.* (1978).

In particular the electrocortical changes were continuously (every 1 or 5 min) computerized as previously described (Nisticò *et al.*, 1981) in order to get continuous information on total voltage power as well as on preselected bands of ECoG frequency (0–3; 3–6; 6–9; 9–12 and 12–16 Hz). The time constant (0.03) was short enough to reduce the number of artefacts (HF cut-off was 5.3 Hz). The spectrum power was plotted and the integrated energy signals were expressed as μV^2 per s.

In some animals, for electromuscular (EMG) recording, stainless-steel wires (200 μm diameter) were inserted into the right and left occipital muscles. In order to record EMG activity each rat was connected to an 8 channel EEG polygraph (OTE BIOMEDICA).

To quantify changes of total voltage power and of preselected bands of frequency induced by clonidine or other compounds, the area (expressed in mm^2) under the curve corresponding to plotted total voltage values during 60 min periods after each compound was integrated by means of a Commodore computer and the percentage changes of the integrated area in comparison to the same interval area during pretreatment period were calculated according to the 'trapezoidal rule' (Tallarida & Murray, 1981). To reduce inter-animal variations of baseline electrocortical activity and of single frequency band the percentage changes following drug treatment were compared to the values of the corresponding period before treatment using Student's unpaired (two tailed) t test.

Microinfusion of the same volume of the vehicle lacked effects on behaviour and electrocortical activity. The number of experiments done to obtain each result is shown in parentheses.

Drugs

The drugs were used as their hydrochloride salts: clonidine (Boehringer-Ingelheim, Germany), yohimbine (Sigma St Louis, MO, U.S.A.), prazosin (Pfizer Inc., New York, U.S.A.), phentolamine (Ciba-Geigy, Basle, Switzerland), methoxamine (a gift from Burroughs Wellcome and Co., London). Prazosin was gently heated until it dissolved, all other drugs were easily dissolved in pyrogen-free twice distilled water.

Results

Intraventricular microinjection

The microinfusion of clonidine (9.4, 18.8, 37.6 and 75 nmol) into the third cerebral ventricle induced behavioural and electrocortical slow-wave sleep (SWS) within 5 to 10 min after the start of the infusion and lasting approximately 30–180 min depending on the dose (Table 1) (at least 6 experiments for each dose). During this sleep, the rats showed a periodic increase of total voltage power predominantly in the 0–3, 3–6 and 6–9 Hz frequency bands and a marked reduction in muscle tone; sensory stimuli were able to produce behavioural and phasic electrocortical arousal. Lower doses of clonidine given into the third cerebral ventricle did not affect behaviour and electrocortical activity.

Microinfusion into the locus coeruleus

In comparison to the third cerebral ventricle route, much lower doses of clonidine (0.19, 0.28 and 0.56 nmol) were required to produce behavioural and electrocortical SWS (Figure 1) after unilateral microinjection of clonidine into the locus coeruleus (at least 8 experiments for each dose). In particular, a significant ($P < 0.01$) increase in total voltage power and 0–3, 3–6, 6–9 and 9–12 Hz frequency bands was obtained. These phenomena started within 1–2 min and lasted for different amounts of time depending on the dose (Table 1); in addition, muscular atonia was evoked. Interestingly, the increase in total voltage power occurred initially in the ipsilateral hemisphere

Table 1 Effects of clonidine on electrocortical (ECoG) total voltage power in rats

Brain Region	*Dose clonidine* (nmol)	*Number of experiments*	*Control period*	*1 h after clonidine*	*2 h after clonidine*	*Duration of ECoG SWS* (min)
Third ventricle	9.4	6	88.9 ± 6.46	107.6 ± 6.25*	86.7 ± 9.29	29.5 ± 5.3
	18.8	7	102.7 ± 6.59	129.4 ± 7.82*	100.4 ± 8.47	66.3 ± 10.9
	37.6	8	94.7 ± 6.62	136.8 ± 4.97**	131.9 ± 5.22**	137.4 ± 18.7
	75	8	104.8 ± 5.95	171.0 ± 6.78**	168.8 ± 7.63**	176.6 ± 29.1
Locus coeruleus	0.19	10	74.8 ± 5.05	89.4 ± 6.64	76.1 ± 10.2	25.2 ± 6.1
	0.28	8	105.1 ± 7.72	143.6 ± 6.47**	104.2 ± 8.21	58.3 ± 9.7
	0.56	8	85.6 ± 5.25	124.7 ± 6.12**	121.7 ± 7.12*	129.6 ± 19.3
Locus coeruleus bilaterally	0.056	8	81.8 ± 5.56	134.3 ± 6.8**	133.6 ± 5.9**	165.1 ± 27.5
Dorsal hippocampus	9.4	7	96.5 ± 10.07	99.8 ± 9.27	97.2 ± 10.92	
	18.8	6	101.1 ± 8.14	96.9 ± 11.24	99.4 ± 11.41	
	37.6	6	104.1 ± 10.11	105.0 ± 9.58	101.2 ± 13.2	12.1 ± 4.6
	75	4	97.6 ± 9.57	122.1 ± 9.25*	100.1 ± 10.2	27.3 ± 7.7
	150	6	101.3 ± 8.65	133.8 ± 8.16*	101.2 ± 9.5	
Ventral thalamus	0.56	4	99.6 ± 8.48	101.7 ± 9.35	100.2 ± 10.11	
Anterior thalamus	0.56	4	102.9 ± 10.98	105.6 ± 10.59	103.1 ± 9.17	
Amygdaloid complex	0.56	6	99.1 ± 11.8	101.5 ± 10.61	104.9 ± 12.1	
Frontal cortex	0.56	6	102.5 ± 8.96	115.1 ± 13.22	110.9 ± 7.99	
Sensimotor cortex	0.56	6	115.6 ± 12.18	113.2 ± 11.83	111.2 ± 12.13	

The results are presented as mean values ± s.e.mean of ECoG total voltage power during the control period, 1 and 2 h after clonidine microinjection into several brain regions. Significant differences between control groups and clonidine-treated groups are denoted: *$P < 0.05$ and **$P < 0.01$ (Student's t test). SWS = slow-wave sleep.

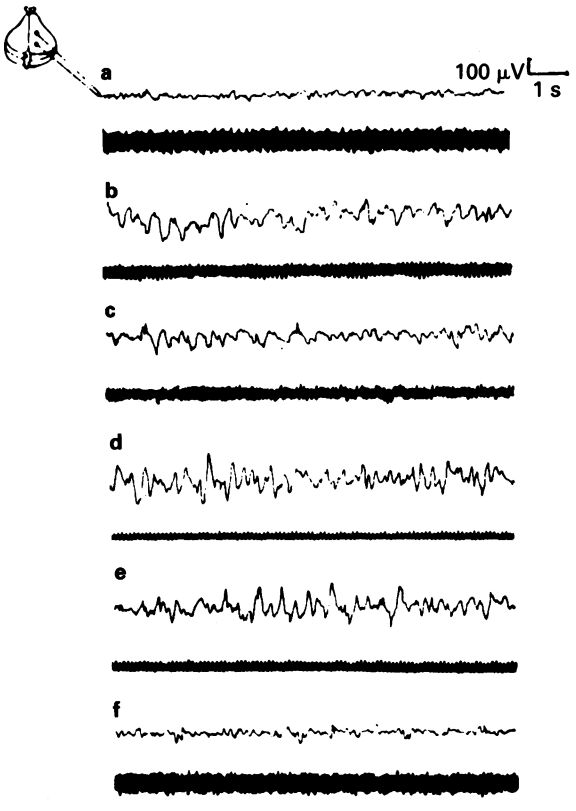

Figure 1 Effects of a single microinfusion into the locus coeruleus of clonidine (0.28 nmol) on electrocortical and electromyographic activity. (a) Control electrocortical and electromyographic activity; (b), (c), (d) and (e) electrocortical slow waves and decrease of electromyographic activity at 5, 30, 60 and 80 min, respectively, after clonidine. (f) Return of ECoG and electromyographic activity to baseline values 100 min after clonidine.

and then within 1–3 min diffused to the contralateral hemisphere.

Bilateral microinjection of clonidine (0.056 nmol) into the locus coeruleus was as effective as unilateral injection of 0.28 nmol (8 experiments) in producing behavioural and electrocortical slow-wave sleep (Figure 2). When the injection of clonidine was more than 1.5 mm distant from the locus coeruleus no sedation occurred.

In comparison to the third cerebral ventricle route, equimolar doses of clonidine (9.4, 18.8 and 37.6 nmol) given into the dorsal hippocampus were almost ineffective in inducing behavioural and electrocortical slow-wave sleep (at least 6 experiments for each dose). Only after larger doses of clonidine (75 and 150 nmol) a slight and short-lasting behavioural and electrocortical sedation occurred (Table 1) (at least 4 experiments for each dose). In addition, clonidine (0.56 nmol) given into the ventral or anterior thalamus, into the amygdaloid complex or onto the frontal or sensimotor cortex produced no changes in behavioural and ECoG activity (Table 1) (4–6 experiments for each area studied).

Effects of α-adrenoceptor antagonists on overt sedation and on ECoG spectrum power changes induced by clonidine

The effects of a pretreatment (15 min before) with α-adrenoceptor antagonists given intracerebroventricularly or into the locus coeruleus on overt sedation and ECoG spectrum power changes induced by clonidine microinjection into the same sites are shown in Figure 3 (at least 4–6 experiments for each antagonist and dose). Phentolamine, an α_1- and α_2-adrenoceptor antagonist (10 and 20 nmol into the locus coeruleus or 20 and 40 nmol into the third cerebral ventricle), or yohimbine, a selective α_2-adrenoceptor antagonist (1.3 and 2.6 nmol into the locus coeruleus or 5.2 and 10.4 nmol into the third cerebral ventricle), was able to prevent behavioural and electrocortical sleep induced by clonidine (Figure 3). In contrast, prazosin, administered intracerebroventricularly (40 nmol) or into the locus coeruleus (5 nmol), was unable to antagonize the behavioural and electrocortical changes induced by clonidine.

In addition, pretreatment with yohimbine (2.56 μmol kg^{-1} i.p. 30 min before) was able to antagonize both behavioural and electrocortical changes induced by clonidine administered either intracerebroventricularly (18.8, 37.6 and 75 nmol) or into the locus coeruleus (0.28 and 0.56 nmol).

Effects of yohimbine

Yohimbine (5.2 and 10.4 nmol) given into the third cerebral ventricle (at least 6 experiments for each dose) induced arousal, an increase in locomotor and exploratory activity, behavioural stimulation, stereotyped movements (sniffing, chewing and licking), tachypnoea which started within 5 min after the injection and lasted 60 to 180 min depending on the dose. Behavioural stimulation was accompanied by a decrease in total voltage power, in the 3–6, 6–9 Hz, and sometimes in the 9–12 Hz frequency bands (Table 2). Yohimbine (5.2 and 10.4 nmol) given into the dorsal hippocampus (4–6 experiments for each dose) produced no significant signs of behavioural and ECoG excitation, although a slight and short-lasting behavioural stimulation, increase in locomotor and exploratory activity was seen (Table 2).

Lower doses of yohimbine (1.3 and 2.6 nmol) were

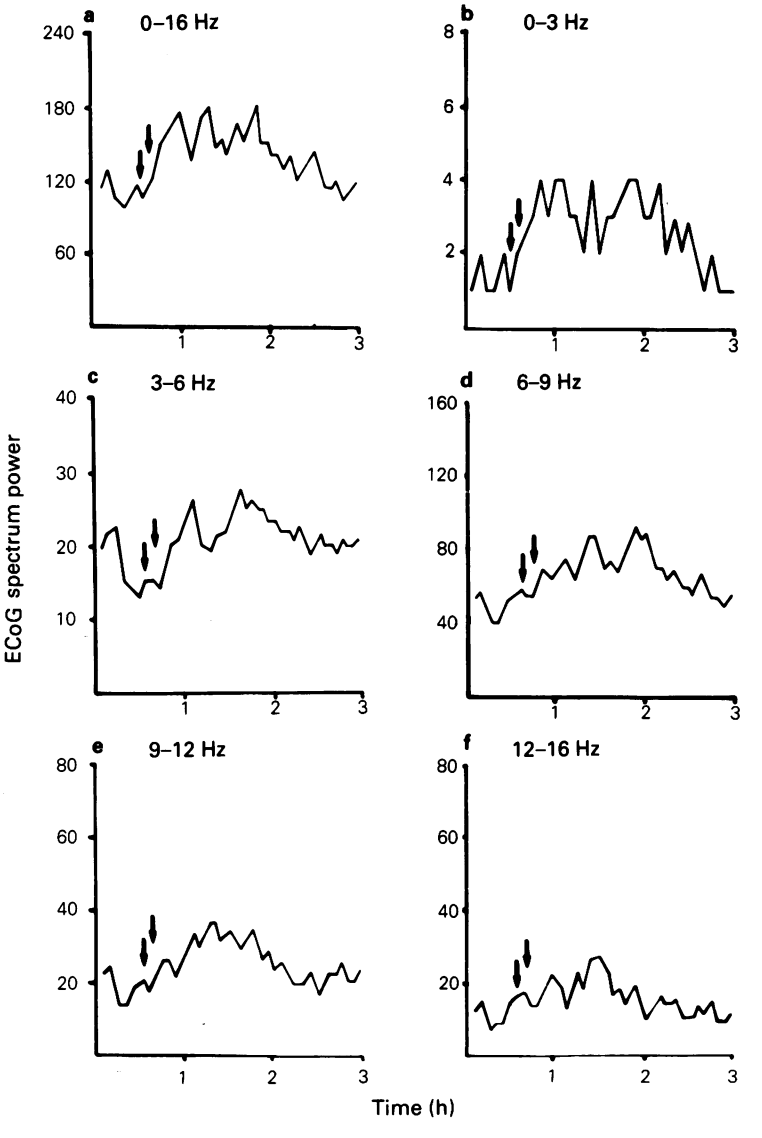

Figure 2 Effects of a bilateral microinjection of clonidine (56 pmol) into the locus coeruleus on electrocortical (ECoG) spectrum power. Ordinates show the voltage power expressed in μV^2 per s, abscissae show time. Note the significant increase in (a) total and (b) 0–3, (c) 3–6, (d) 6–9 and (e) 9–12 Hz voltage power. The slight increase in the 12–16 Hz band (f) was not statistically significant.

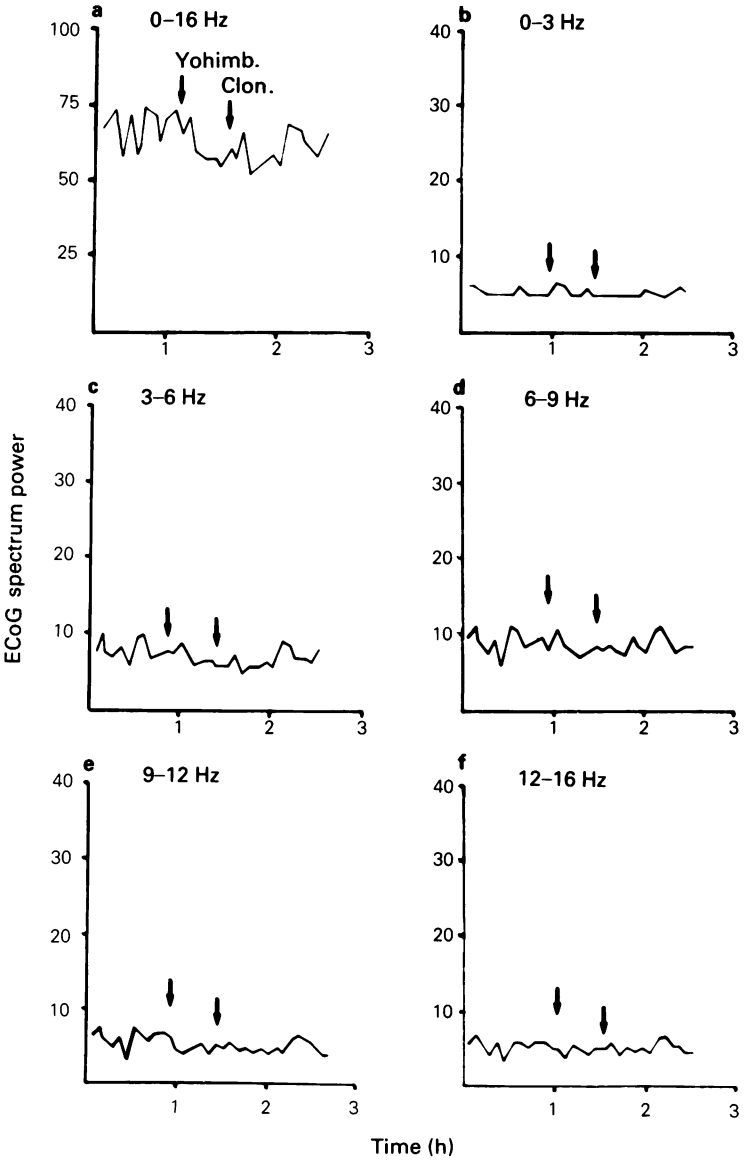

Figure 3 Effects of pretreatment with yohimbine (Yohimb; 1.3 nmol, 15 min before) into the locus coeruleus on electrocortical (ECoG) spectrum power changes induced by clonidine (Clon; 0.56 nmol). Ordinates show the voltage power expressed in μV^2 per s, abscissae show time. (a) Total, (b) 0–3, (c) 3–6, (d) 6–9, (e) 9–12 and (f) 12–16 Hz frequency bands.

Table 2 Effects of α-adrenoceptor agonists and antagonists on electrocortical (ECoG) total voltage power in rats

Brain region	*Drug and dose* (nmol)	*Number of experiments*	*Control period (1 h)*	*1 h after drug*	*2 h after drug*	*Duration of ECoG desynchronization* (min)
	Yohimbine					
Third ventricle	5.2	6	104.7 ± 13.1	80.5 ± 11.6	101.3 ± 12.7	57.9 ± 11.7
	10.4	7	99.2 ± 11.7	68.8 ± 9.81	71.4 ± 10.3	176.8 ± 31.5
Dorsal	5.2	4	97.1 ± 10.4	86.7 ± 9.64	95.7 ± 11.1	15.7 ± 5.23
hippocampus	10.4	6	101.4 ± 13.7	85.9 ± 12.3	102.28 ± 10.9	21.4 ± 7.11
Locus coeruleus	1.3	6	101.5 ± 9.2	78.1 ± 8.6	99.4 ± 10.4	59.6 ± 12.9
	2.6	8	97.6 ± 8.9	69.2 ± 8.5*	70.4 ± 8.3*	161.4 ± 30.6
	Phentolamine					
Third ventricle	20	6	98.5 ± 10.5	82.0 ± 13.3	100.3 ± 12.1	41.7 ± 9.6
	40	6	100.9 ± 10.2	76.4 ± 9.4	83.3 ± 10.6	84.1 ± 23.7
	60	6	89.3 ± 9.6	63.3 ± 8.5	76.5 ± 9.7	112.5 ± 29.8
Locus coeruleus	10	6	103.6 ± 8.8	84.9 ± 8.3	102.1 ± 9.8	43.6 ± 11.5
	20	6	96.5 ± 8.3	66.7 ± 8.5*	82.3 ± 8.7	79.1 ± 21.3
	Methoxamine					
Third ventricle	40	4	99.7 ± 10.3	96.5 ± 11.7	97.3 ± 10.9	
	80	4	103.9 ± 11.7	104.1 ± 12.5	100.2 ± 12.7	
Locus coeruleus	10	6	91.9 ± 9.6	95.4 ± 11.2	93.7 ± 10.5	
	20	6	97.8 ± 11.4	93.6 ± 9.2	94.5 ± 9.6	
	Prazosin					
Third ventricle	20	6	106.1 ± 12.1	108.6 ± 11.5	105.3 ± 10.1	
	40	6	101.6 ± 11.4	107.4 ± 9.3	105.1 ± 9.2	
Locus coeruleus	5	4	98.4 ± 13.2	104.9 ± 9.1	102.2 ± 10.7	
	10	4	93.2 ± 9.7	103.2 ± 8.9	101.7 ± 11.6	

The results are presented as mean values ± s.e.mean of ECoG total voltage power during the control period, 1 and 2 h after drug microinjections into several brain areas. Significant differences between control groups and drug treated groups are denoted: $*P < 0.05$ (Student's t test).

necessary, when it was infused into the locus coeruleus (at least 6 experiments for each dose), to induce behavioural and electrocortical desynchronization (Figure 4) similar to that observed after intracerebroventricular administration (5.2 and 10.4 nmol) (Table 2).

Effects of phentolamine

Infusion of phentolamine (20, 40 and 60 nmol) into the third cerebral ventricle induced behavioural and electrocortical changes similar to those already observed with yohimbine. Lower doses of phentolamine (10 and 20 nmol) were required after microinfusion into the locus coeruleus to produce effects similar to those evoked after intraventricular administration (Table 2) (6 experiments for each dose).

Effects of methoxamine and prazosin

Methoxamine, an α_1-adrenoceptor agonist, injected into the third cerebral ventricle (40 and 80 nmol) or locus coeruleus (10 and 20 nmol) did not affect the behavioural and the electrocortical activity of the animals studied (Table 2) (4–6 experiments for each dose and brain area). No significant changes in behavioural and electrocortical activity occurred following administration of prazosin into the third cerebral ventricle (20 and 40 nmol) or into the locus coeruleus (5 and 10 nmol) (Table 2) (4–6 experiments for each dose and brain area).

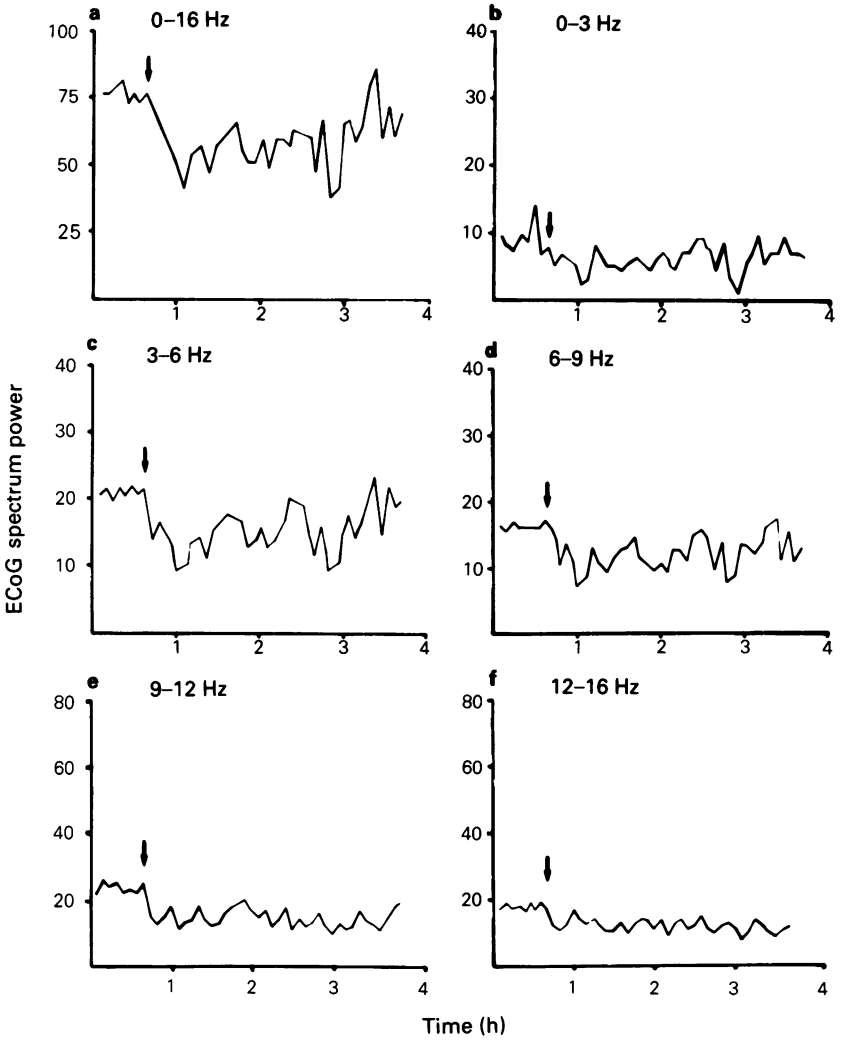

Figure 4 Effects of a single microinjection into the locus coeruleus of yohimbine (2.6 nmol) on electrocortical (ECoG) spectrum power. Ordinates show the voltage power expressed in μV^2 per s, abscissae show time. Note the significant fall in (a) total and in (c) 3–6, (d) 6–9 and (e) 9–12 Hz voltage power.

Discussion

The present experiments confirm that clonidine possesses powerful behavioural sedative effects accompanied by electrocortical SWS and an increase in the total and in the low frequency bands of power. The most sensitive area from which clonidine-induced soporific effects were evoked is represented by the locus coeruleus, an area containing the largest clusters of noradrenaline (NA)-containing cell bodies in the CNS (Dahlström & Fuxe, 1964). No behavioural sedation and ECoG synchronization was induced by clonidine after microinfusion in areas where there is a large projection of NA-containing axonal terminals (hippocampus, amygdaloid complex, thalamus, frontal and sensimotor cortex). This indicates that activa-

tion by clonidine of α_2-adrenoceptor sites, located on the noradrenergic terminals reached by the drug after its microinfusion in these specific areas of the brain, is not able to produce sleep, in contrast to the profound soporific effects obtained from the locus coeruleus. Autoradiographic studies have provided evidence for the existence of a high density of α_2-adrenoceptor binding sites in the locus coeruleus (Young & Kuhar, 1980). It seems obvious that stimulation of α_2-adrenoceptors located on the membrane of NA-containing cell bodies in the locus coeruleus leads to a decrease in the activity of ascending noradrenergic pathways diffusely projecting to subcortical and cortical areas. The inhibition of noradrenergic inputs projecting to the cortex may well be the consequence of a hyperpolarization of locus coeruleus neurones mediated by α_2-adrenoceptors, as shown by extracellular single-unit (Svensson *et al.*, 1975) and by intracellular recordings *in vivo* after microiontophoretic application or systemic administration of clonidine (Aghajanian & Vander Maelen, 1982; Depoortere, 1985).

In addition, evidence exists showing that in behaving rats, in experimental conditions in which spontaneous discharge of NA-containing locus coeruleus neurones was recorded, this was lowest during slow-wave sleep and highest during waking (Aston-Jones & Bloom, 1981b). In contrast, in cats inactivation of the locus coeruleus neurones by specific localized cooling (Cespuglio *et al.*, 1982) or electrolytic lesions of the dorsal noradrenergic bundle ascending from the locus coeruleus (Petitjean *et al.*, 1975) are followed by an increase in both slow-wave sleep and paradoxical sleep. Furthermore, it has also been shown that electrolytic, neurotoxic lesions of catecholaminergic systems in the CNS as well as inhibition of catecholamine synthesis is followed by behavioural and ECoG sedation and a decrease in waking activity (see Monti, 1982). Moreover, electrical stimulation of the locus coeruleus of cats is followed by ECoG and behavioural arousal (Koella, 1978; 1984); similar results were obtained in macaques (Redmond *et al.*, 1976). This evoked arousal response could be due to the release of NA at the cortical level (Tanaka *et al.*, 1976).

The specificity of α_2-adrenoceptors in mediating clonidine-induced behavioural and ECoG sleep is confirmed by experiments showing that selective antagonists at α_2-adrenoceptors were effective in preventing clonidine effects. An involvement of α_1-adrenoceptors at the level of the locus coeruleus neurones in the control of sleep-arousal mechanism has to be ruled out since α_1-adrenoceptor agonists, such as methoxamine and selective antagonists of α_1-adrenoceptors, i.e. prazosin, were unable to affect behaviour and ECoG activity after their microinjection into the third cerebral ventricle or into the locus coeruleus. However, α_2-adrenoceptors on locus coeruleus neurones seem to exert a tonic inhibition on cortical arousal, since we found that the microinfusion of yohimbine, an α_2-adrenoceptor antagonist, into this area led to behavioural stimulation and ECoG desynchronization. Similar effects have been observed in rats after intraventricular administration of yohimbine (Zebrowska-Lupina & Kleinrok, 1973). Comparable results were also obtained in chicks using other α_2-agonists, i.e. guanabenz and guanfacine, and α_2-antagonists (Nisticò *et al.*, 1979; Marley & Nisticò, 1983; Rotiroti *et al.*, 1983). The role of noradrenergic mechanisms in the control of sleep-arousal has been, for a long time, a matter of controversy since the systemic administration of drugs enhancing catecholaminergic transmission gives rise to behavioural stimulation and ECoG desynchronization, whereas the direct microinjection of catecholamines in the cerebral ventricles of different animal species produces sleep, although stimulatory effects have also been found (see Marley & Stephenson, 1972). Such contrasting effects are not necessarily contradictory. They may be explained by the fact that after intraventricular administration, catecholamines and other adrenoceptor agonists seem to reach more easily and to act predominantly at the locus coeruleus neurones decreasing the level of activity of noradrenergic systems projecting to the cortex. In contrast, the systemic administration of drugs potentiating noradrenergic transmission via different mechanisms, e.g. L-DOPA, (+)-amphetamine or inhibitors of NA re-uptake, may activate predominantly postsynaptic adrenoceptors only at the cortical level and produce ECoG desynchronization (see Marley & Nisticò, 1983; Koella, 1984). The stimulatory role of α_1-adrenoceptors at the level of the cerebral cortex is also supported by microiontophoretic studies in which the application of methoxamine on rat cortical neurones was shown to produce an increase in neuronal firing (Bradshaw *et al.*, 1981).

The lack of effect of methoxamine after intracerebroventricular administration may be due to the fact that from the third cerebral ventricle the drug does not reach the areas in which noradrenergic terminals project (i.e. cerebral cortex) and where a higher density of postsynaptic α_1-adrenoceptors occurs.

Since intraventricular administration of noradrenaline or locus coeruleus stimulation has been shown to increase γ-aminobutyric acid (GABA) release and to decrease acetylcholine release from the cerebral cortex (Moroni *et al.*, 1980), it seem plausible that the effects on sleep-arousal mechanisms, due to manipulations affecting noradrenergic mechanisms in the locus coeruleus, may be mediated through changes in other transmitters released at the cortical level.

Further experiments are necessary to ascertain the role of β-adrenoceptors in arousal mechanisms (see

Monti, 1982), although β-adrenoceptor antagonists were found to reduce the cortical effects of locus coeruleus stimulation (Koella, 1978; 1984).

In conclusion, the present experiments show that behavioural and ECoG SWS induced by clonidine is due to activation of α_2-adrenoceptors located on the noradrenergic cell bodies of the locus coeruleus with consequent inhibition of noradrenergic mechanisms. In addition, the stimulatory effects elicited by microinfusion of α_2-adrenoceptor antagonists into the locus coeruleus suggest that in rats α_2-adrenoceptors are tonically active during the light-period to reduce the level of arousal.

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