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ALTERED RESPONSIVENESS OF CENTRAL α_2-ADRENOCEPTORS IN AGING

G. NISTICO' and G.B. DE SARRO

Istituto di Farmacologia, Facoltà di Medicina di Catanzaro, Università degli Studi di Reggio Calabria, Italy

Summary. - *The behavioural and ECoG spectrum power effects of clonidine, and yohimbine, an agonist and an antagonist at α_2-adrenoceptors, after their unilateral microinfusion into the rat* locus coeruleus *(LC) in young (50-70 days old) and old (13-15 months old) rats were studied. Clonidine (0.09, 0.19, 0.28 and 0.56 nmol) microinfused into the LC of young rats induced dose-dependent behavioural and ECoG slow wave sleep (SWS) with a significant increase in total voltage power and in the lower frequency bands. In contrast, yohimbine (1.3 and 2.6 nmol) infused into the LC of young rats produced ECoG desynchronization and a significant decrease in total voltage power. In contrast to young rats, clonidine (0.19 and 0.28 nmol) given into the LC did not affect behaviour and ECoG spectrum power in old rats. However, after higher doses of clonidine (0.56 and 1.2 nmol) a small and short-lasting period of behavioural and ECoG SWS was still evident. Similarly, in old rats yohimbine, at a dose (1.3 nmol) which was stimulatory in young animals, did not significantly affect behaviour and ECoG spectrum power. Higher doses of yohimbine (2.6 and 5.2 nmol) were required to induce behavioural and ECoG changes similar to those observed with lower doses of yohimbine in young rats. The present experiments suggest that there is a decreased responsiveness of LC α_2-adrenoceptors in old rats.*

Riassunto (Alterata responsività degli α_2-recettori adrenergici nell'invecchiamento). - *Gli effetti comportamentali ed elettrocorticografici (ECoG) della clonidina e della yohimbina, rispettivamente un agonista ed antagonista dei recettori α_2-adrenergici, sono stati studiati dopo microinfusione del* locus coeruleus *(LC) di ratti giovani (50-70 giorni) e ratti anziani (13-15 mesi). La microinfusione di clonidina (0,09, 0,19, 0,28 e 0,56 nmol) nel* locus coeruleus *del ratto giovane produce uno stato di sedazione comportamentale ed elettrocorticografico associato ad un aumento della potenza totale dello spettro elettrocorticografico e delle bande di frequenza più basse. Tali fenomeni sono apparsi dose-dipendenti, Al contrario, la microinfusione nel LC di yohimbina (1,3 e 2,6 nmol) produce nel ratto giovane un aumento della attività motoria ed esplorativa associato a desincronizzazione ECoG e significativo decremento della potenza totale dello spettro ECoG. La microinfusione di clonidina (0,19 e 0,28 nmol) a dosi analoghe a quelle risultate sedative e/o soporifiche nel ratto giovane, non ha prodotto significative modificazioni comportamentali ed elettrocorticografiche nel ratto anziano. Dosi di clonidina più elevate (0,56 e 1,2 nmol) hanno prodotto nell'animale anziano uno stato di sedazione comportamentale ed ECoG simile a quello osservato nel ratto giovane. Similmente, una dose di yohimbina (1,3 nmol) che era risultata stimolatoria nel ratto giovane non ha influenzato significativamente il comportamento e l'attività ECoG nel ratto anziano. Dosi più alte di yohimbina (2,6 e 5,2 nmol) hanno prodotto nell'animale anziano un quadro comportamentale ed ECoG simile a quello descritto con dosi più basse nell'animale giovane. I presenti esperimenti suggeriscono che nel ratto anziano esiste una ridotta responsività dei recettori α_2-adrenergici localizzati nel LC.*

Introduction

The effects of aging on behaviour and electrocortical (ECoG) activity have been widely studied in several animal species [1]. ECoG alterations in old rats consist of spontaneous, asymptomatic, bilaterally symmetrical and synchronous bursts of epileptic-like spikes occurring prevalently from 6.8 to 9.4 Hz, the animals being immobile in a freezing-like state. These typical behavioural and ECoG changes can be stopped abruptly with sound stimulation and no correlation between these changes and audiogenic seizures was observed [2]. It has been suggested that these behavioural and ECoG alterations may be due to changes of neuronal membrane fluidity and consequently to alterations in the neurotransmission in the brain [3-6].

The *locus coeruleus* (LC) is a densely packed cell group located in the dorsal pons which contains approximately half of all noradrenergic neurones in the rat brain [7-9]. The main ascending and descending noradrenergic pathways in the brain originate from the LC [10, 11]. In addition, it has been recognised for a long time that the LC is a crucial area in the control of the sleep-waking cycle [12-17]. Recently, we have reported that the α_2-adrenoceptor agonist and antagonist, clonidine and yohimbine, microinfused into the LC, are able to affect sleep/arousal mechanisms in rats [18]. Until now no data are available in the literature concerning the behavioural and electrocortical changes produced by pharmacological manipulations of LC neurons in old animals.

We report here that male Sprague-Dawley rats display, as they get older, a decrease of responsiveness of the LC neurones to clonidine and yohimbine.

Methods

Adult young (50-70 days old; 200 ± 20.2 g) and old (13-15 months old; 670 ± 30.6 g) male Sprague Dawley rats were purchased from Charles River, (Calco, Como, Italy) and housed in stable conditions of humidity (60 ± 5%) and temperature (22 ± 2 °C). They were fed with standard diet and had water *ad libitum*. Animals were mantained on 12 h a light and dark cycle (lights on 6 h 00 min-18 h 00 min, off 18 h 00 min-6 h 00 min).

Rats were stereotaxically implanted under chloral hydrate anaesthesia (400 mg/kg, i.p.; Carlo Erba, Milan) with permanent stainless steel guide cannula (25 gauge), which had an angle of 5° from the vertical and the tip 2 mm from the LC all coordinates were modified from the atlas coordinates of Paxinos and Watson [19] (AP= 1.1. mm posterior to lambda, L= 0.5 mm lateral to the midline, H= 5.7 mm ventral to the skull surface).

After surgery a minimum of 48 h was allowed before experiments were carried out. All experiments were performed beginning at approximately 10 h 00 min. Freely moving rats were microinjected via an injector cannula (28 gauge) which extended approx. 2 mm below the tip of guide cannula.

Electrocortical (ECoG) activity was recorded via 4 chronically implanted steel screw electrodes inserted onto each fronto-parietal cortex (young rats: 2 mm behind the bregma and ± 2 mm laterally to the midline; old rats: 2.2 mm behind the bregma and ± 2.2 mm laterally to the midline) by a Stoelting stereotaxic frame. All electrodes and the injection guide cannula were anchored to the skull by acrylic dental cement. Electrocortical activity was recorded by means of an 8 channel EEG machine (OTE Biomedica, Florence). For statistical purpose, the quantification of total voltage power (0.25-16 Hz) and of individual frequency bands (0.25-3; 3-6; 6-9; 9-12; 12-16 Hz) was carried out by using a Berg Fourier analyzer (OTE Biomedica, Florence) according to the method of Bricolo *et al.* [20]. ECoG spectra power were obtained by averaging spectra derived from 5 min ECoG epochs and the integrated energy signals were expressed as $\mu V^2/s$; the time constant (0.03s) was short enough to reduce the number of artifacts (HF cut-off= 5.3 Hz).

Each recording session (5 h duration) started 60 min after the electrodes were connected.

The animals, placed individually in transparent cages (35 × 35 × 25 cm), were allowed 60 min prior to drug administration to acclimatize to the new environment. The behavioural changes and their onset and duration were recorded after drug injection. In particular, two independent observers followed gross behavioural changes consisting in eyes open or closed, locomotor activity, possible stereotyped movements, squatting posture and also they noted whether the rats concomitantly to ECoG changes were alert, drowsy or sleepy. Each animal was used only once.

Post-mortem histological examination confirmed the location of the guide cannula. Only animals in which the location of the injection site was confirmed histologically were used in the analysis of behavioural and ECoG data.

To quantifiy changes of total voltage power and of preselected bands of frequency induced by clonidine, yohimbine or saline, 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 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» [21]. To reduce inter-animal variation of baseline electrocortical activity and of single frequency bands the percentage changes following drug-treatment were compared to the values of corresponding period before treatment. In order to verify whether experimental groups were homogenous, data of young and old rats were previously analysed using a one-way analysis of variance followed, where appropiate, by analysis with the Student's t-test. ECoG spectrum data are presented in terms of means ± the standard errors of the variation of total voltage power and of preselected bands of frequency. The percentage changes following drug-treatment were compared to the value of the corresponding period before treatment using paired Student's t-test.

Drugs: Clonidine hydrochloride (Boehringer-Ingelheim, Germany) and yohimbine hydrocloride (Sigma St Louis, Mo, USA) were infused into the *locus coeruleus* using a 5 µl Hamilton syringe, connected by a teflon tube to an injector cannula. The drug was infused in a volume of 0.5 µl at a rate of 0.2 µl min and the cannula left *in situ* for a further 1 min. Each animal was treated only once. Control infusions were

carried out with the same volume of saline which has been used to dissolve clonidine or yohimbine hydrochloride and lacked effects on overt behaviour and electrocortical spectrum power activity.

Result

Effect of clonidine

The microinfusion of clonidine into the LC of young rat (0.09, 0.19, 0.28 and 0.56 nmol) induced behavioural and electrocortical slow-wave sleep (SWS) within 1-2 min after the infusion and lasting approximately 13-14 min depending on the dose (Table 1) (at least 8 experiments for each dose).

During sleep, rats showed periodic increase of total voltage power and predominantly in 0.25-3, 3-6, 6-9 and sometimes 9-12 Hz frequency bands. In addition, muscular atonia was observed; sensory stimuli were able to produce behavioural and phasic electrocortical arousal.

In old rats the dose of 0.28 nmol did not significantly affect the behavioural and electrocortical activity, while the highest dose (0.56 nmol) induced a significantly shorter lasting (approx. 30 min) behavioural and ECoG SWS (Table 1 and Fig. 1) (n = 8 for each dose).

In order to get similar effects as those observed with clonidine (0.56 nmol) in young rats, a higher dose of clonidine (1.2 nmol) was required (n = 10 for each dose). The behavioural and ECoG sleep and the increase in total voltage power and in the preselected frequency bands evoked by clonidine infusion were significantly less marked and shorter lasting in old than in young rats (Table 1 and Fig. 1).

Effects of yohimbine

Yohimbine (1.3 and 2.6 nmol) given into the LC of young rats (n = 8 for each dose) induced arousal, an increase in locomotor and exploratory activity,

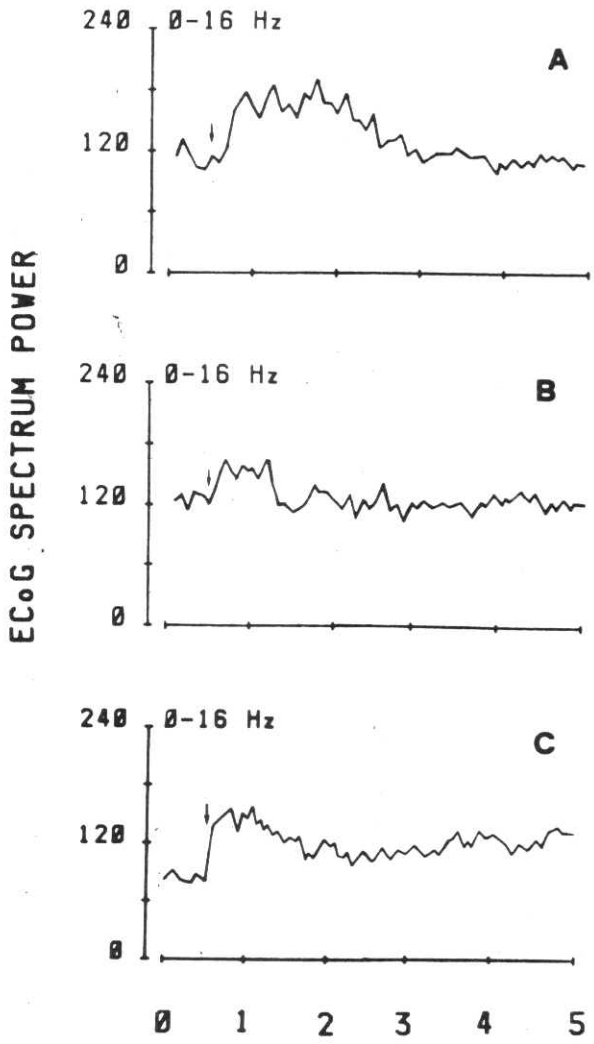

Fig. 1. - Effects of a single microinfusion into the *locus coeruleus* of clonidine (0.56 nmol) on electrocortical (ECoG) spectrum power of a young (A) and an old (B) rat, whilst in (C) are reported the changes on ECoG spectrum power observed after microinfusion of clonidine (1.2 nmol) into the *locus coeruleus* of an old rat. Ordinates show the voltage power expressed in $\mu V^2/s$, abscissae show the time expressed in hours. Note the mild ECoG spectrum power changes observed after clonidine (0.56 nmol) in old rat (B).

Table 1. - *Effects of microinfusion of clonidine into the* locus coeruleus *on electricortical (ECoG) total voltage power in young and old rats*

Clonidine (nmol)	Number of experiments	Age (months)	Control period	ECoG total voltage power ($\mu V^2/s$)	
				1 h after clonidine	2 h after clonidine
0.09	8	2	101.7 ± 4.75	109.8 ± 6.52	102.1 ± 6.25
0.19	8	2	94.8 ± 3.26	113.3 ± 4.61	98.4 ± 7.02
0.28	10	2	102.1 ± 6.79	139.5 ± 5.72 **	104.6 ± 7.72
0.56	9	2	96.5 ± 5.56	148.6 ± 6.05 **	136.4 ± 6.25 *
0.19	8	13-15	99.2 ± 6.51	101.6 ± 5.92	99.3 ± 6.5
0.28	8	13-15	100.3 ± 6.14	104.7 ± 6.27	101.2 ± 6.1
0.56	10	13-15	97.6 ± 7.95	117.5 ± 7.37	99.3 ± 7.29
1.2	10	13-15	101.2 ± 7.14	144.4 ± 5.66 **	133.7 ± 7.63 *

The results are presented as mean values ± SE of the ECoG total voltage power during the control period, 1 and 2 h after clonidine microinjection in young rats. Significant differences between control groups and clonidine treated groups are denoted * $p < 0.05$ and ** $p < 0.01$ (paired Student's t-test).

Table 2. - *Effects of microinfusion of yohimbine into the* locus coeruleus *on electricortical (ECoG) total voltage power in young and old rats*

Yohimbine (nmol)	Number of experiments	Age (months)	Control period	ECoG total voltage power ($\mu V^2/s$)	
				1 h after yohimbine	2 h after yohimbine
1.3	8	2	98.9 ± 6.72	76.8 ± 7.8	95.4 ± 8.4
2.6	8	2	102.1 ± 7.46	72.9 ± 8.3 *	74.3 ± 8.5 *
1.3	6	13-15	94.8 ± 7.05	80.9 ± 10.1	95.6 ± 8.7
2.6	8	13-15	101.5 ± 7.73	76.4 ± 8.9	96.4 ± 9.1
5.2	8	13-15	98.6 ± 6.25	69.5 ± 8.6 *	72.4 ± 8.2 *

The results are presented as mean values ± SE of the ECoG total voltage power during the control period, 1 and 2 h after yohimbine microinjection in young and old rats. Significant differences between control groups and yohimbine treated groups are denoted * $p < 0.05$ (paired Student's t-test).

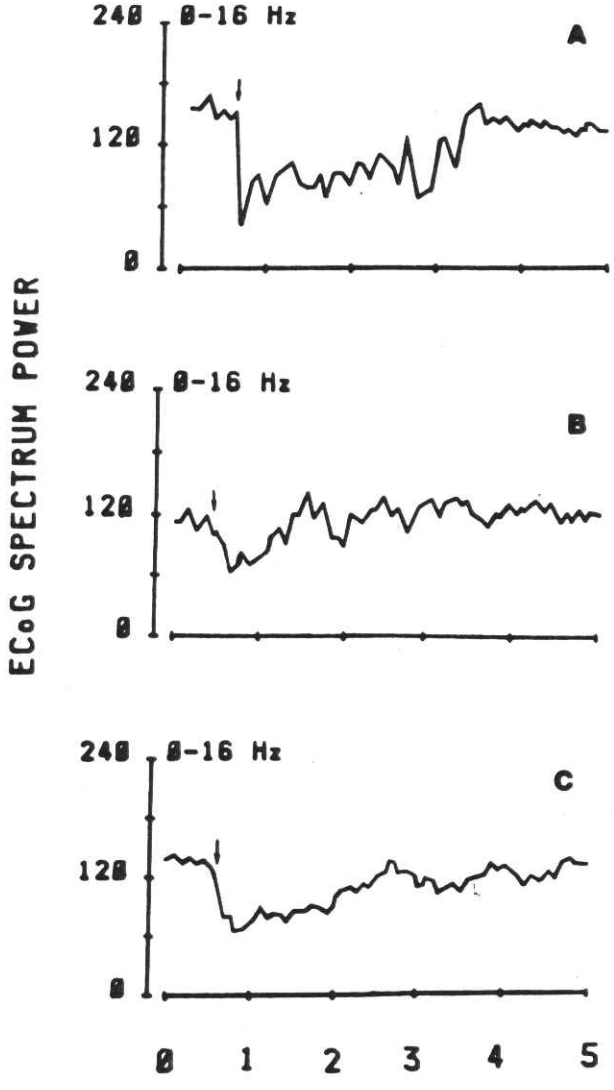

Fig. 2. - Effects of a single microinjection into the *locus coeruleus* of yohimbine (2.6 nmol) on electrocortical (ECoG) spectrum power of a young (A) and an old (B) rat, whilst in (C) are reported the changes an ECoG spectrum power observed after microinfusion of yohimbine (5.2 nmol) into the *locus coeruleus* of an old rat. Ordinates show the voltage power expressed in $\mu V^2/s$, abscissae show the time expressed in hours. Note the mild ECoG spectrum power changes observed after yohimbine (2.6 nmol) in old rat (B).

stereotyped movements (sniffing, chewing and licking), tachypnoea which started within 3 min after the injection and lasted 60-160 min depending on the dose. Behavioural stimulation was accompanied by a significant decrease in total voltage power, in the 3-6, 6-9 and sometimes in the 9-12 Hz frequency bands (Table 2).

In contrast to young animals, the microinfusion of yohimbine (1.3 and 2.6 nmol) into the LC of old rats did not significantly affect the behavioural and electrocortical activity (Table 2 and Fig. 2, n = 6 for each dose). A higher dose of yohimbine (5.2 nmol) was necessary in old rats to produce a behavioural and ECoG pattern similar to that observed after microinfusion of yohimbine (2.6 nmol) in young rats (n = 6-8 for each dose).

Behavioural and ECoG changes seen after yohimbine in old rats were much less marked and shorter lasting than those reported in young ones (Table 2 and Fig. 2).

Discussion

De Sarro *et al.* [18] previously reported that bilateral microinfusion of clonidine at very low doses (0.056 nmol) produced in young rats behavioural sleep accompanied by ECoG synchronization, increase in ECoG total voltage power and power in the lower frequency bands, whereas yohimbine produced behavioural arousal and ECoG desynchronization. The present experiments show that in older rats there is a decreased sensitivity to both clonidine and yohimbine. In fact, in old rats small doses of clonidine and yohimbine were ineffective in changing behaviour and ECoG activity, whereas only after higher doses of clonidine a small and shorter lasting behavioural sleep with an increase in ECoG total voltage power occurred. The reason for such reduced responsiveness to the α_2-adrenoceptors agonist and antagonist in old rats is not known at the present time, although one can hypothesize that behavioural and ECoG alterations occurring with aging may affect the behavioural and bioelectric responses to

neurotransmitters. Thus it is not clear whether in old rats there is a decrease in number of α_2-adrenoceptors in the LC and/or a decrease in their affinity for agonist and antagonist drugs. This phenomenon may not be specific to α_2-adrenoceptors in the brain. Evidence from a number of studies has suggested an impairment of the neurotransmission in the brain in old rats [4, 6, 22, 24]. In addition, it has been reported that β-adrenoceptor responsiveness is reduced in aged rats [25] and that the number of α_1- and β-receptors is decreased in the cerebral cortex of 25 month-old rats as compared to 3 month-old rats [26]. However, little is known about changes in central α_2-adrenoceptors in aging, although in the vas deferens reduced sensitivity of prejunctional α_2-adrenoceptors was found in old rats [27]. Other authors have reported an age-dependent reduction of the number of α_2-adrenoceptors in human platelets [28] and rabbit brain [29].

A possible mechanism that might underly some of the age-related change in receptor function is an alteration in lipid composition and fluidity of neuronal membranes. The reversal of age-related decrease in membrane mediated responses brought about by chronic treatment with phosphatidylserine, as recently reported by De Sarro *et al.* [30, 31], is consistent with such a mechanism.

In conclusion, the present experiments provide evidence that there is a decreased responsiveness of LC α_2-adrenoceptors in old rats.

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