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Differential Clonidine Effects on EEG Following Lesions of the Dorsal and Median Raphe Nuclei in Rats

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DYR, W., W. KOSTOWSKI, B. ZACHARSKI AND A. BIDZINSKI. *Differential clonidine effects on EEG following lesions of the dorsal and median raphe nuclei in rats.* PHARMACOL BIOCHEM BEHAV **19**(2) 177–185, 1983.—The effects of clonidine on EEG activity and gross behavior were studied in rats with electrolytic lesions of the median (MR) and dorsal (DR) raphe nuclei. Lesioned animals showed significant depletion in forebrain serotonin concentrations. Clonidine (0.1 mg/kg and 0.2 mg/kg IP) produced synchronization in cortical EEG pattern and markedly increased alpha and theta activities in unlesioned animals. Clonidine treatment resulted also in a sedative response. In MR lesioned rats clonidine effect upon EEG was significantly reduced and, additionally, sedative response was not seen. On the other hand clonidine effect on EEG was markedly increased in rats with lesioned DR. These results are discussed on the basis of possible interaction between serotonergic and noradrenergic neurons in the brain.

Raphe nuclei Clonidine EEG

CLONIDINE, the selective agonist of α_2 adrenoceptors in the brain depresses the release of noradrenaline (NE) from noradrenergic nerve terminals because of stimulation of this receptor subgroup [17, 26, 27]. Clonidine has been also reported to inhibit the bioelectrical activity of NE neurons of the locus coeruleus (LC), probably through activation of α_2 adrenoceptors located on the cell bodies of NE neurons [28]. These mechanisms are related to the well known depressive affects of clonidine such as inhibition of locomotor and exploratory activities, suppression of avoidance behavior and suppression of self-stimulation in laboratory animals [3, 4, 6, 13, 16]. In addition to its behavioral effects clonidine has been reported to induce synchronization in cortical EEG pattern in rats, cats and rabbits [5]. Both behavioral and EEG actions of clonidine were prevented by the administration of yohimbine and piperoxan, the antagonists of α_2 adrenoceptors and were, therefore, attributed to stimulation of this receptor subgroup by clonidine [3,4]. Numerous investigators believe that clonidine may serve as a tool for investigation of interaction between NE neurons and other neurotransmission systems. It has been also supposed that this drug may provide a new approach to investigation of central adrenergic receptors [3].

Most workers assume that brain NE neurons are under the inhibitory influence of serotonergic (5-HT) neurons. It is known that lesions (either electrolytic or chemically-induced) of the raphe nuclei, the main source of both ascending and descending 5-HT fibres increase the concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG), the major metabolite of NE in the brain [13]. This finding and other data not described here indicate that brain NE neurons, particularly those located within the locus coeruleus and forming so-called dorsal NE bundle are tonically inhibited by 5-HT neurons of the raphe system [14,15]. When 5-HT system is lesioned or the activity of 5-HT neurons is reduced due to pharmacological treatment the NE neurons became hyperactive [15]. More recently evidence has accumulated which suggests that lesions of brain 5-HT neurons attenuated behavioral effects of clonidine [16,30]. Our previous studies have shown that both electrolytic and 5,6-dihydroxytryptamine-induced lesions of the midbrain raphe nuclei antagonized suppression of gross activity and suppression of avoidance acquisition by clonidine in rats [16]. It seemed therefore interesting to study the role of the raphe nuclei in clonidine-induced synchronization. In this study we investigated the effects of electrolytic lesions of dorsal and median raphe nuclei on clonidine action in rats with chronically implanted cortical electrodes.

METHOD

Animals

Male Wistar rats weighing 180–200 g at the beginning of the experiment were kept singly in wire mesh cages

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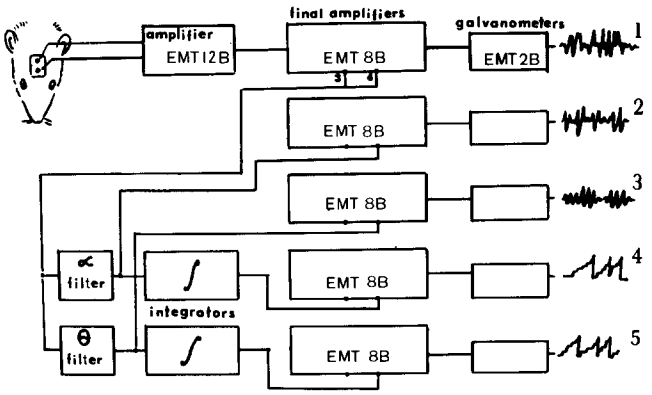

FIG. 1. Block diagram of EEG processing system. 1—original EEG, 2—alpha filter output, 3—theta filter output, 4—integrated alpha activity, 5—integrated theta activity. Integrated activities recorded as charge-discharge cycles at the integrator output.

(30×30×45 cm) in normal laboratory conditions (natural day-night cycle, temperature 20–22°C) with free access to food and tap water.

Brain Lesions

Lesions were made under chloral hydrate anaesthesia by use of Stoelting stereotaxic apparatus and stainless steel electrode (0.25 mm in diameter) insulated except for the tip. The anodal current (2 mA) was delivered through the electrode for 10 sec (with an indifferent needle electrode inserted into the tail). The following areas were lesioned according to the coordinates given in the König and Klippel rat brain atlas [10]: nucleus raphe medianus (MR): A=0.4 mm, L=0.0 mm, H=−2.6 mm and nucleus raphe dorsalis (DR): A=0.4 mm, L=0.0 mm and H=−0.6 mm. Sham operated animals were prepared by inserting the electrode 1.0 mm dorsal to the MR or DR yet not passing a current.

Implantation of Cortical Electrodes

Seven days after lesions the animals were implanted (under chloral hydrate anaesthesia) with two cortical electrodes (silver wire 1.0 mm in diameter) placed epidurally over left and right frontal cortical areas. The electrodes were fixed to the skull with screws and dental acrylic cement. After implantation rats were housed singly in wire mesh cages and 7 days were allowed to pass between surgery and the initiation of EEG recording.

EEG Recording

The cortical EEG pattern was recorded always at the same period of day (10.00 a.m.–1.00 p.m.) without removing animals from their home cages. The electrodes were connected to the EEG apparatus by use of special plugs, long cable and counter-weighted slip-ring assembly thereby permitting free movement within the cage. EEG recordings and their processing were performed by an arrangement showed in Fig. 1. EEG signal picked up by two electrodes was fed to the input of Universal Amplifier EMT-12B of the polygraph Mingograph 81, Siemens-Elema, Sweden. Amplified output from EMT-12B was fed to the input of Final Amplifier EMT-8B, thus the ink-jet galvanometer EMT-2B recorded original EEG signal in the first channel. Simultaneously amplified EEG signal picked-up from the output (pin 3) of EMT-8B was fed to inputs of alpha and theta filters (8–14 Hz and 4–8 Hz respectively with 24 dB/octave roll-off, manufactured by ITR, Warszawa, Poland). Outputs from the filters were fed to the driver and output stages (pin 4) of EMT-8B and were recorded by galvanometers of channels 2 and 3 respectively. Filtered EEG containing alpha and theta activities were fed into integrators (time constant $\tau=50$ msec). Outputs from integrators were fed to pin 4 of EMT-8B and were recorded by galvanometers of channel 4 and 5. The integrated alpha and theta activites served as measures for investigated phenomena and as input data for further processing.

Administration of Clonidine

Clonidine HCl (Boehringer-Ingelheim, FRG) was dissolved in 0.9% NaCl and injected IP at 0.1 and 0.2 mg/kg^{-1} in a volume of 1 ml/kg^{-1}. Control animals received 0.1 ml of 0.9% NaCl.

Histology and Biochemical Determinations

After the completion of the experiment the animals were sacrificed by decapitation, their brains were quickly removed and dissected by precollicular section (caudally to the hypothalamus) to remove the forebrain and brain stem. The brain stems of lesioned animals were checked histologically for lesion location and size after fixation in 10% formalin and staining with hematoxyllin and eosin. Biogenic amine levels were measured in the whole forebrains of lesioned and sham lesioned animals. The extraction and fluorimetric determination of brain areas were carried out basically according to the Haubrich and Denzer [8] with modification of Korf and Sebens [11].

Statistics

EEG data were evaluated using KS (Kolmogorow and Smirnow) test (two sample) [20] while biochemical data were analysed by means of the Student t-test, two tailed.

RESULTS

Location of Lesions

Histological examination showed that lesions involved mainly the MR or the DR and in some rats partially destroyed the superior cerebellar penduncle and the mesencephalic reticular formation (when MR was lesioned) or small portions of the central gray substance (when DR was destroyed). Location of the lesions is showed in Fig. 2.

Biochemical Examination

Rats with lesions involving the MR showed marked decrease in the forebrain 5-HT concentration (almost 50 percent). Lesions of the DR decreased forebrain 5-HT level approximately by 30 percent. No significant changes in 5-hydroxyindole acetic acid (5-HIAA) as well as NE concentrations were observed (Table 1).

Evaluation of Clonidine Effects on EEG

Clonidine given in doses 0.1 and 0.2 mg/kg^{-1} markedly synchronized cortical EEG pattern as evidenced by increase

TABLE 1

FOREBRAIN 5-HT, 5-HIAA AND NE CONCENTRATIONS IN RATS WITH LESIONED THE MEDIAN NUCLEUS (MR) AND THE DORSAL RAPHE NUCLEUS (DR)

Experimental group	n	Forebrain concentrations ng/g ± SEM		
		5-HT	5-HIAA	NE
Sham lesion	8	503.4 ± 26.0	629.5 ± 65.0	436.1 ± 22.3
MR lesion	6	262.7 ± 33.0*	538.5 ± 22.0	429.0 ± 34.3
DR lesion	6	349.6 ± 19.9*	603.7 ± 90.2	422.7 ± 90.2

*$p<0.01$ In respect to sham lesioned animals, n—number of animals.

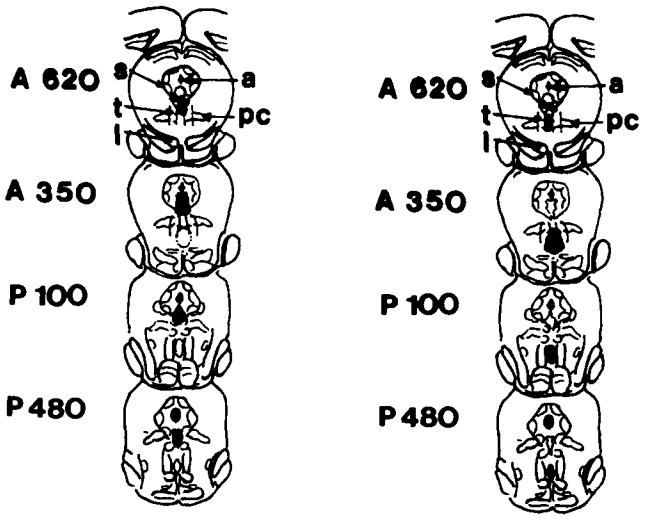

FIG. 2. Placement and size of lesions (black areas) involving dorsal raphe nucleus (DR) (left panel, composite of 8 lesions) or median raphe nucleus (MR) (right panel, composite of 7 lesions). Numbers—distance in μm anterior (A) and posterior (P) to the zero plane of the König and Klippel stereotaxic atlas [10]. Abbreviations: a—cerebral aqueduct, l—fasciculus longitudinalis, pc—pedunculus cerebellaris superior, s—substantia grisea centralis, t—tractus tectospinalis.

in alpha and theta activities (Fig. 3). Visual observations revealed that animals treated with clonidine were sedated and usually crouched in the corner of the cage, particularly after administration of higher dose (0.2 mg/kg^{-1}). Both EEG and behavioral effects of clonidine lasted for about 120–150 min. Animals with lesioned MR showed reduced EEG response to clonidine (Figs. 4, 5) and were apparently less sedated (visual observation showed that rats were normally active and walked within the cage). On the other hand EEG effects of clonidine were markedly potentiated in rats with lesioned DR (Figs. 5, 7). No changes in animals gross behavior after clonidine administration to DR lesioned rats have been observed.

Lesion of the MR by itself produced desynchronization in the cortical EEG pattern in approximately 50 percent of animals. Lesioned rats were apparently more active behaviorally than sham lesioned and DR-lesioned animals. No change in gross behavior was observed in untreated rats with DR lesion.

DISCUSSION

The data obtained herein are consistent with the observations of Florio *et al.* [5] which have shown cortical EEG synchronization in rats treated with clonidine. In accordance with these authors we observed that a dose of 0.1 mg/kg had the same effect as 0.2 mg/kg.

Lesions of the raphe nuclei by itself changed the basel cortical EEG pattern. These results, however, were not quantitatively measured in this study. It is well known that

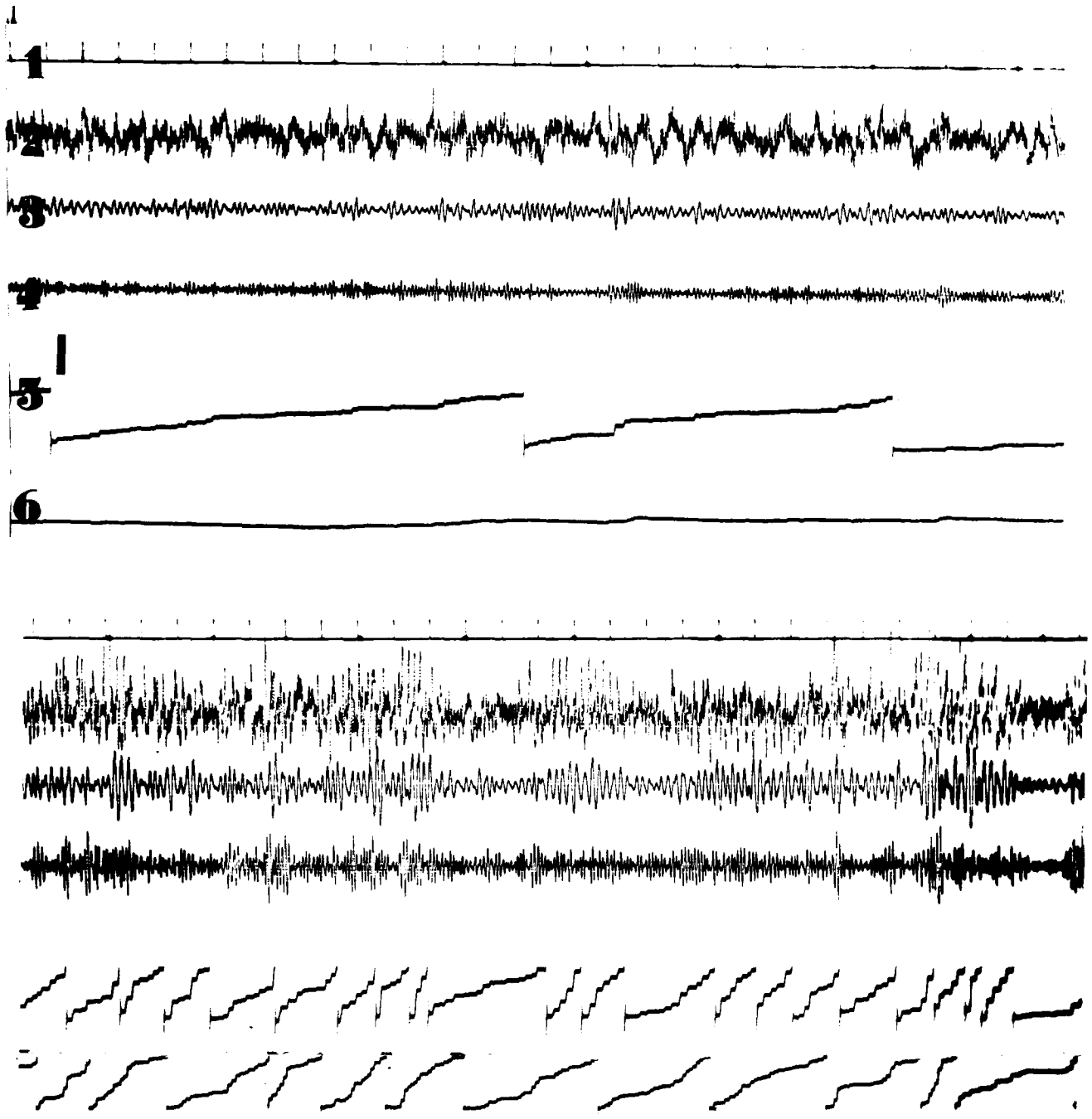

FIG. 3. Effect of clonidine on EEG pattern of the sham lesioned rat (prepared by inserting the electrode tip 1.0 mm dorsal to the MR yet not passing the current). Control resting pattern (upper tracing) compared with pattern recorded 15 min after clonidine at 0.1 mg/kg (lower tracing). 1—time calibration, 1 sec, 2—original EEG, 3 and 4—ouputs of alpha and theta filter respectively, 5 and 6—integrated alpha and theta activities respectively (charge-discharge cycles at the integrator output). Vertical bar—100 μV.

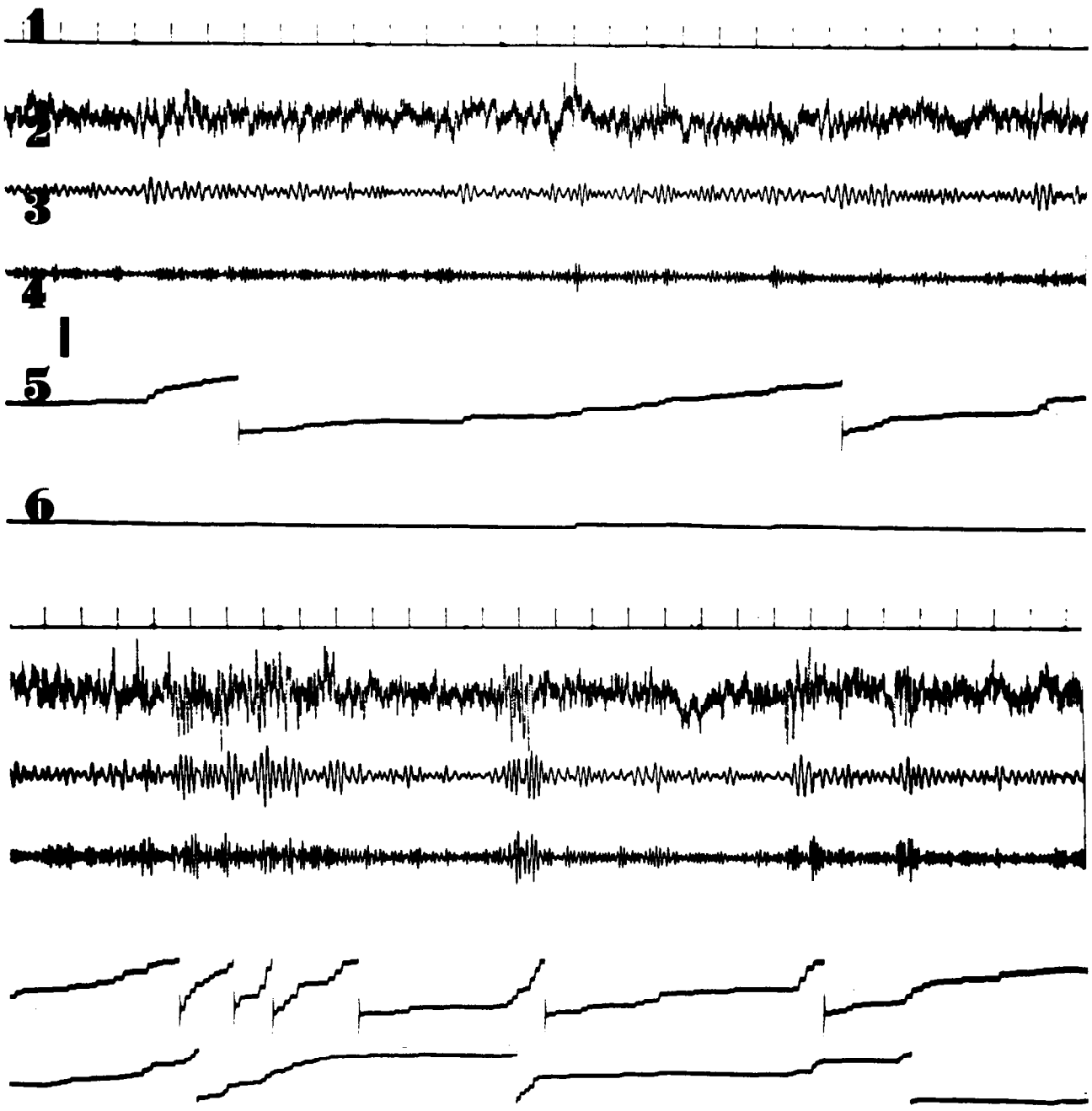

FIG. 4. Effect of clonidine on EEG of the MR lesioned rat. Upper tracing—sample of control EEG pattern recorded one week after MR lesion, the lower tracing was recorded in the same animal 15 min after clonidine (0.1 mg/kg). Clonidine produced only slight synchronogenous effect. For other explanation see Fig. 3.

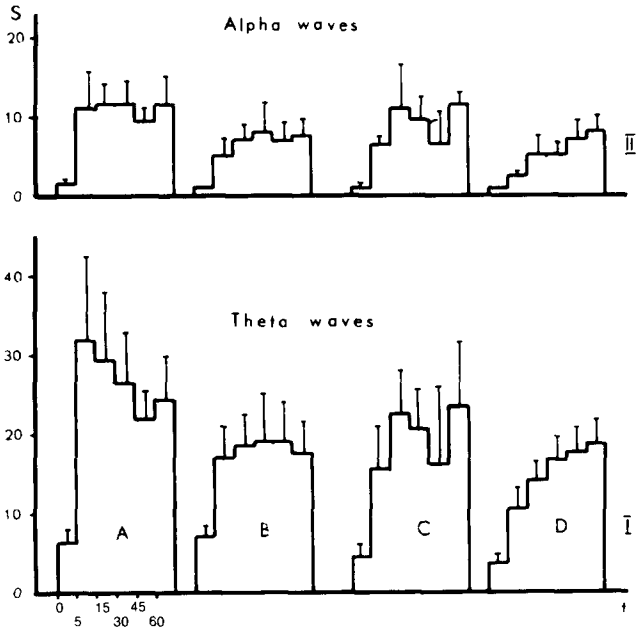

FIG. 5. Effect of clonidine on alpha and theta EEG activities in MR lesioned rats. Vertical scale-integrated activities measured as a mean number of charge-discharge cycles per min. Each column represents mean activity±SEM (from 6–8 animals) measured during 1 min, 5 and 15 minutes after clonidine administration and then every 15 minutes over a period of 60 min *t*-time after injection of clonidine (0—one minute segment preceding the injection). A—sham lesioned treated with clonidine 0.1 mg/kg, B—MR lesioned treated with clonidine 0.1 mg/kg, C—sham-lesioned treated with clonidine 0.2 mg/kg, D—MR-lesioned treated with clonidine 0.2 mg/kg. The differences between block A and B and between C and D were statistically significant ($p<0.05$).

MR lesions produce cortical EEG desynchronization in rats [12,31], however little attention has been devoted to EEG changes after lesion of the DR. Our result showing synchronogenous effect of DR lesion is compatible with finding described previously by Yamamoto *et al.* [31]. Interestingly enough, previous studies from different laboratories found that electrical stimulation of the DR caused arousal pattern in the EEG while stimulation of the MR resulted rather in cortical synchronization [31]. From these and other data (see below), the midbrain raphe nuclei could not be regarded as functionally homogenous system.

The present data indicate that 5-HT depleting lesions of the MR attenuated synchronogenous action of clonidine. On the other hand animals with lesioned DR showed increased response to clonidine as evidenced by marked increase in both alpha and theta activities. This finding provides further evidence supporting the existence of substantial differences between two nuclei at the midbrain level. It is possible that anatomical specificity of ascending projections from these nuclei account for this phenomenon. It is known that DR appears to contribute more fibres to the cortex (particularly to temporoparietal areas), amygdala and the basal nuclei. The projection to the hippocampus, frontal cortex and entorhinal cortex is delivered mostly from the MR [2]. It is noteworthy that substantial pharmacological differences between DR and MR have been found by numerous investigators. For example, analgesic effects of morphine were significantly reduced in the rats with lesioned MR but not DR [24]. Selective lesions of the DR but not the MR increased so-called muricide (mouse-killing) behavior in chronically isolated rats [29]. Lesions of the MR have been reported to induce rather much stronger locomotor excitation than lesions of the DR [7, 9, 19, 25].

As mentioned previously, our recent study found that in rats with 5,6-hydroxytryptamine-induced lesion of the MR clonidine failed to produce its normal depressive effect upon avoidance acquistion and gross behavior [16]. Electrolytic lesions of the DR or MR have been also reported to attenuate depressive action of clonidine on the open field behavior in rats [30]. Since in the present study no quantitative analysis of behavior has been made we prefer to avoid to draw definite conclusion from simultaneous behavoral observations. Our impression, however, was that clonidine failed to produce behavioral depression in rats with lesioned MR.

This study together with the data previously published [15,16] suggests that destruction of the MR system indeed may produce a long-lasting stimulation of NE neurons, thereby decreasing their sensitivity to clonidine. This state-

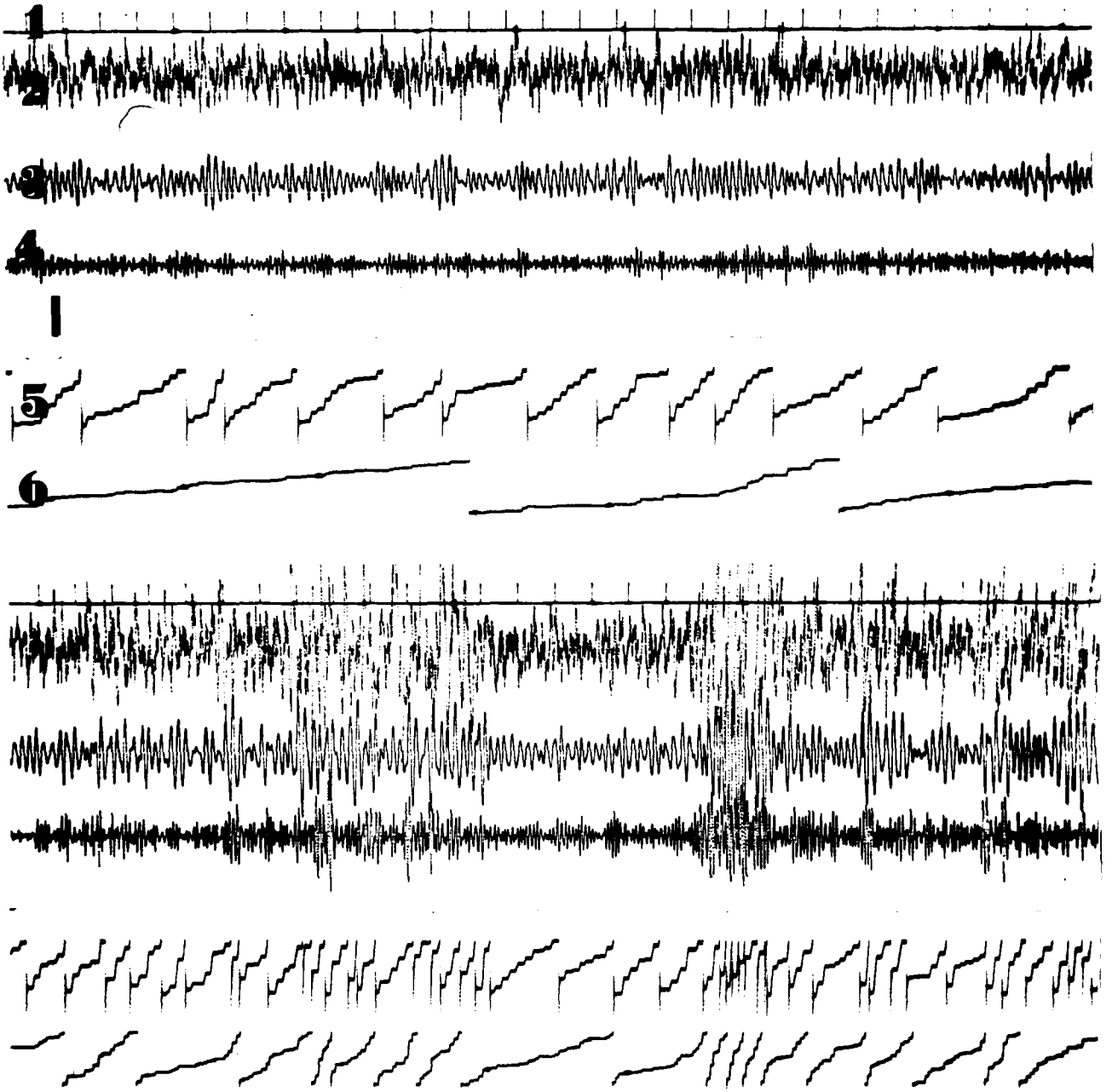

FIG. 6. Effect of clonidine on EEG of the DR lesioned rat. Upper tracing-sample of control EEG pattern recorded one week after DR lesion, the lower tracing was recorded in the same rat 15 min after clonidine (0.1 mg/kg). Note synchronized resting pattern in DR lesioned animal and marked synchronogenous effect of clonidine. For other explanation see Fig. 3.

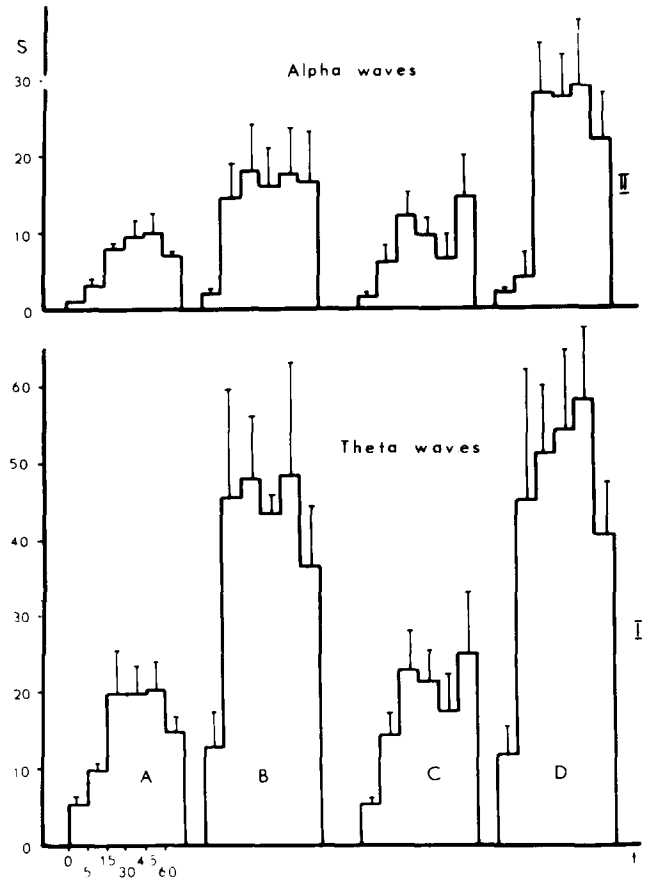

FIG. 7. Effect of clonidine on alpha and theta EEG activities in DR lesioned rats. A—sham lesioned rats treated with clonidine 0.1 mg/kg, B—DR lesioned rats treated with clonidine 0.1 mg/kg, C—sham-lesioned rats treated with clonidine 0.2 mg/kg, D—DR lesioned treated with clonidine 0.2 mg/kg. Mean values±SEM from 8–9 animals. The differences between A and B and C and D were statistically ($p<0.01$ for alpha activity and $p<0.001$ for theta activity). For other explanations see Fig. 5.

ment is, however, not true for the lesion of the DR system since this lesion exerts opposite influence upon clonidine action.

Behavioral effects of clonidine have been assumed to be suitable for use as a model for the detection of drug interaction with α_2 adrenoceptors in the brain [3]. The synchronogenous effect of clonidine seems to be also associated with stimulation of α_2 adrenoceptors since it can be easily reversed by antagonists of these receptors [3,4]. It is of interest to note that clonidine reduced bioelectrical activity of NE neurons of the locus coeruleus [28], the area considered to be involved with mechanisms of cortical EEG desynchronization [1, 18, 22].

If one assumes that clonidine-induced EEG synchronization is due to stimulation of α_2 adrenoceptors it seemy likely that lesions of the raphe nuclei influence clonidine effects by modulation of activity of central NE neurons. The mechanism of this interaction, however, is different for MR and DR and cannot be simply explained on the basis of tonic inhibitory action of 5-HT neurons on NE neurons. It is therefore, possible that mechanisms other than direct 5-HT/NE interaction might play a role in the phenomena described in this study.

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