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Action of Psychotropic Drugs upon pO_2 in the Lateral Amygdala and Pontine Reticular Formation during the Sleep-Wakefulness Cycle

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MONTI, J. M. AND R. VELLUTI. *Action of psychotropic drugs upon pO_2 in the lateral amygdala and pontine reticular formation during the sleep–wakefulness cycle.* PHARMAC. BIOCHEM. BEHAV. 2(6) 763–767, 1974. – A study was carried out of the actions of nitrazepam, haloperidol and pentobarbital upon pO_2 of nuclei amygdalae lateralis and reticularis pontis caudalis during the sleep–wakefulness cycle in cats with chronically implanted oxygen cathodes. Nitrazepam at the 0.25 mg/kg dose selectively diminished the pO_2 oscillations in the lateral amygdala. Further, phasic changes depicted during REM sleep in the pontine reticular formation were abolished. After larger doses (0.5–1.0 mg/kg) there was a simultaneous and progressive decrease of the current oscillations in both structures. Haloperidol produced a reduction of pO_2 current waves only after the highest administered dose (2.0 mg/kg), without specificity for any of the structures recorded. Sedative and anesthetic doses of pentobarbital also decreased pO_2 waves amplitude in a dose-response related manner.

Sleep Nitrazepam Haloperidol Pentobarbital Amygdala Reticular formation Oxygen cathode Polarography

SEVERAL approaches have been followed to determine the structures of the CNS involved in the various actions of psychotropic drugs.

They include the study of (1) the effects of intracerebral or systemic drug injection on spontaneous or evoked electrical activity in different areas of the brain and (2) the effects of drug administration on behavioral and EEG responses elicited by electrical or chemical stimulation of various brain centers.

Recently, Velluti *et al.* [20], García-Austt *et al.* [4] and Velluti and Monti [22], utilizing the oxygen cathode characterized the pattern of oscillations of the limbic system and brain stem reticular formation during the sleep–wakefulness cycle. It was shown that pO_2 varied in each structure during the behavioral stages according to a characteristic and fixed pattern.

The reproductiveness of the results induced us to utilize the method to study the site of action of some depressant drugs. We chose three psychotropic drugs [5], an anxiolytic (nitrazepam), a neuroleptic (haloperidol) and an hypnotic (pentobarbital) and determined their actions on the pO_2 of two nuclei, one belonging to the limbic system (nucleus amygdalae lateralis, NAL) and the other to the reticular formation (nucleus reticularis pontis caudalis, NRPC) during the sleep–wakefulness cycle. Those anatomical structures were selected on the basis of previous information assigning to them a role on the regulation of attentional and emotional behavior [8,12].

The results obtained showed that the compounds modified pO_2 differently and in some cases selectively, according to the involved structure.

METHOD

Animals

Five adult cats of both sexes were used in this investigation. In order to control the behavioral state of the animals, electrodes were permanently implanted on the lateral cortex for electrocorticogram (ECoG) and the dorsal neck muscles for electromyogram (EMG). Electrodes were also implanted in two cats in the lateral geniculate nucleus (A 7.0, L 10.5, H +4.0) for the recording of PGO spikes.

Procedure

Changes of brain pO_2 were monitored by means of

[1] We are indebted to Mr. H. Altier for the histological preparations. We are grateful to Roche Laboratories for the nitrazepam (Mogadon®) and to Antia Moll Laboratories for the haloperidol.

platinum oxygen cathodes referred to anodes made of AgCl-Ag wires [2, 3, 20] which were stereotaxically implanted [18] in the NAL (A 11.0, L 12.5, H −6.0) and the NRPC (P 2.0, L 3.0, H −4.0). Ten days after implantation, when fully recovered, the cats were placed in a dimly lighted soundproof isolated box fitted with one-way mirror and recordings made through a multiple strand cable using a Grass 7 polygraph. A constant voltage of −0.6 V was applied to the oxygen cathode and the oxygen reduction-dependent current was obtained through a voltage drop measured across a 270 Ω resistance in the polygraph by means of a low level DC preamplifier, Model 7P1. Recordings were carried out with the low-pass filter of the amplifier set to a cut-off frequency of 3 Hz. In every experiment the oxygen-dependence of the electrodes was tested by changing the oxygen concentration in the cage, replacing air by pure oxygen. Histological verifications of electrode placement were carried out at the end of the experiments.

Each derivative was studied at the following dose levels: nitrazepam 0.125, 0.25, 0.5 and 1.0 mg/kg; haloperidol 0.5, 1.0 and 2.0 mg/kg and pentobarbital sodium 10.0 and 30.0 mg/kg. Control sessions without injection and solvent-injection sessions were interspersed between drug sessions. All drug injections were given intraperitoneally after control recordings lasting 2 hr which included periods of wakefulness, slow wave and REM sleep.

RESULTS

The pO_2 recordings in the NAL and NRPC exhibited characteristic patterns during the sleep–wakefulness cycle.

While the animals were awake (Fig. 5) oscillations with a frequency of 6–9/min and an amplitude of 3–8 nA could be depicted in either nucleus. When the cats entered into the slow wave phase of sleep (Figs. 1, 3 and 4) the pattern of oscillations in NAL shifted to one of high amplitude current waves (20–30 nA) intermingled with smaller waves (8–16 nA) while their frequency remained almost the same. At the level of the NRPC there was a small increase in the frequency and amplitude of the current waves, amounting to 12/min and 16 nA, respectively.

During the REM phase of sleep (Figs. 1 and 2) oscillations in the NAL returned to a pattern similar to that of wakefulness. Conversely, the record of the NRPC was characterized by the presence of high amplitude current waves (18–23 nA) with a frequency of 4/min, with hardly any place left for the previous rhythm. Doses of 0.25–1.0 mg/kg nitrazepam markedly increased cortical fast activity and the number of sleep spindles. pO_2 oscillations were not modified after 0.125 mg/kg of the benzodiazepine derivative (5 experiments), while 0.25 mg/kg (12 experiments) selectively diminished the amplitude of the current waves at the level of the NAL during slow wave and REM sleep (Figs. 1, 2 and 3). In the NRPC the most striking finding was the disappearance of the high amplitude phasic pO_2 oscillating response observed during REM sleep. The electrocortical desynchronization and PGO spikes characteristic of this behavioral stage were still present (Figs. 1 and 2). After 0.5 mg/kg (14 experiments) and 1.0 mg/kg (5 experiments) as dose–response related decrease in the amplitude of the oscillations was observed in both nuclei during slow wave sleep (Fig. 3). With the highest dose this effect was still observed 6 hr later.

Haloperidol did not induce any appreciable change in the ECoG patterns, but significantly modified the sleep cycle, reducing REM sleep time with the highest dose. After 0.5–1.0 mg/kg (9 experiments with each dose) there were no changes in the pO_2 responses. A dose of 2.0 mg/kg (7 experiments) reduced the amplitude of the current waves in both nuclei (Fig. 4) during slow wave sleep. When REM sleep reappeared all these tonic and phasic components were present, including the high amplitude oscillations of the reticular formation.

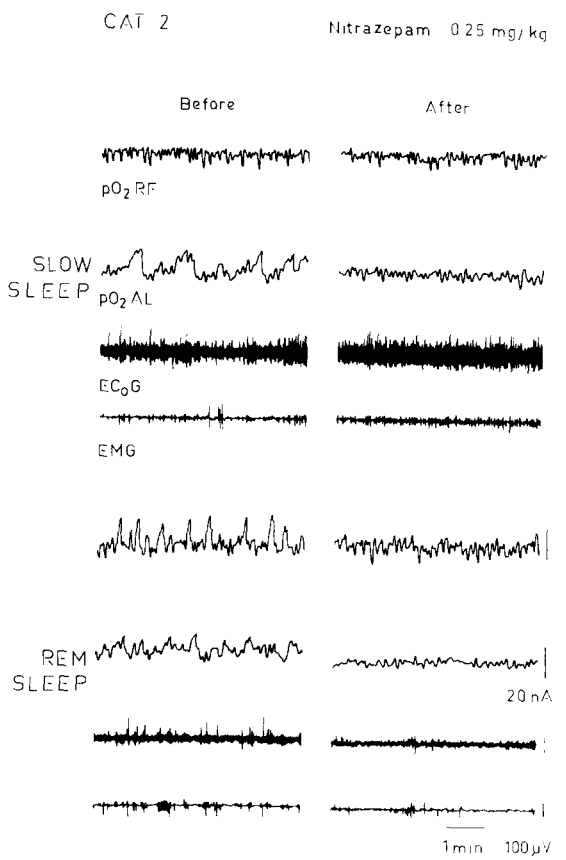

FIG. 1. Effects of nitrazepam (0.25 mg/kg) upon pO_2 current waves in subcortical structures during slow wave and REM sleep. Abbreviations: pO_2 current waves from the nuclei reticularis pontis caudalis (RF) and amygdalae lateralis (AL); ECoG electrocorticogram of the lateral cortex; EMG electromyogram of the neck muscles. Calibration: pO_2 in nanoamperes; ECoG and EMG in microvolts. Time in minutes. The benzodiazepine derivative selectively decreased the amplitude of pO_2 oscillations in the nucleus AL during slow wave and REM sleep and abolished the phasic changes depicted during the desynchronized phase of sleep in the nucleus RF. The changes described during slow wave and REM sleep were recorded 20 and 68 min after nitrazepam, respectively.

Pentobarbital increased the incidence of larger and slower ECoG frequencies, and abolished REM sleep. After 10.0 mg/kg (7 experiments) a decrease in the amplitude of the current waves was depicted in the amygdala and reticular formation, while 30.0 mg/kg (8 experiments) induced an almost complete disappearance of the pO_2 oscillations (Fig. 5).

DISCUSSION

After the administration of the psychotropic drugs, the

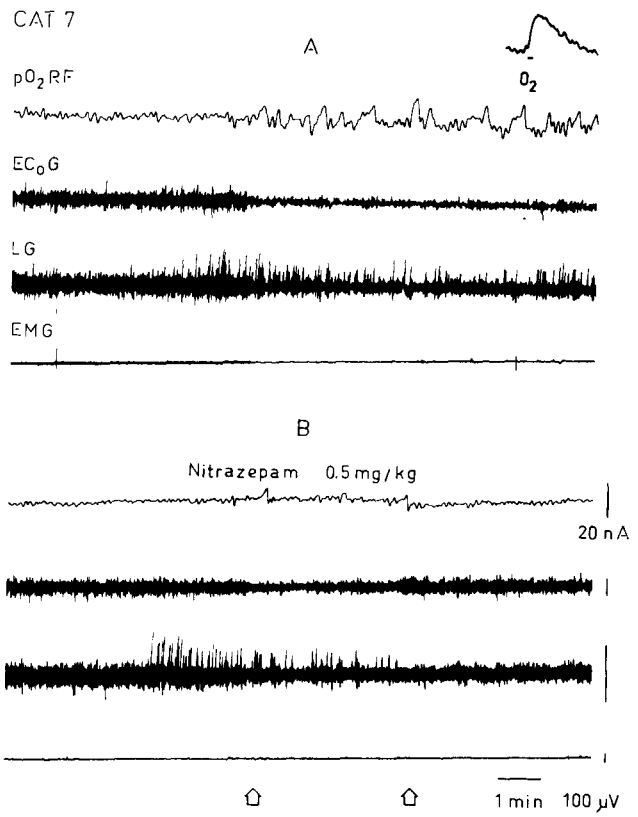

FIG. 2. Effects of nitrazepam (0.5 mg/kg) on pO_2 oscillations upon the nucleus reticularis pontis caudalis during REM sleep. LG electroencephalogram of lateral geniculate nucleus. Others as in Fig. 1. In A, at the beginning of the record, the cat is in slow wave sleep and the oxygen cathode exhibits small oscillations. Subsequently an REM period is shown marked by the activation of the ECoG, the appearance of PGO spikes and a drop of the EMG. Concomitantly with these changes high amplitude oscillations of slower frequency in the RF are observed. During a period of wakefulness, the air of the cage was changed by pure oxygen (O_2), a sudden increase of pO_2 being provoked (Inset). In B the phasic pO_2 changes depicted in the RF during REM sleep (shown between arrows) are almost completely abolished after nitrazepam administration. Conversely, cortical desynchronization and PGO spikes are not modified.

changes observed in the ECoG were similar to those previously described [6, 7, 9].

Most authors agree that the oxygen cathode is actually recording the pO_2 of the extracellular compartment [2,3]. Furthermore, the oxygen concentration in this compartment is a function of the local oxygen-consuming metabolic cellular activity and of the local oxygen-supplying blood flow. The local pO_2 changes during the sleep–wakefulness cycle were regarded by Velluti *et al.* [20] and García-Austt *et al.* [4] as reflecting local neuronal activity, which is assumed to be greatly increased in the NRPC during REM sleep. The amplitude of pO_2 oscillations was selectively diminished in the NAL during slow wave and REM sleep following 0.25 mg/kg nitrazepam. Moreover, the phasic changes depicted during REM sleep in the NRPC were no longer present. Other indicators of this stage of sleep (cortical desynchronization and PGO spikes) remained constant. With higher doses of nitrazepam (0.5–1.0 mg/kg) the amplitude of oscillations diminished in both structures simultaneously. The selectivity of action of nitrazepam (at a particular dose level) on the lateral amygdala suggested by our experiments was also contended by Schallek and Kuehn [14] who observed that the compound selectively increased afterdischarge thresholds in the same structure. Nagy and Decsi [11] microinjecting diazepam into different parts of the CNS also drew the conclusion that the benzodiazepine derivative acts upon the amygdaloid complex. It could be argued that the decreased amplitude of the pO_2 waves after nitrazepam administration is related to a smaller oxygen-supplying blood flow. Current evidence does not support this suggestion since benzodiazepines fail to show effects on myocardial contractility [13]. In addition, systemic blood pressure is not decreased in spite of a depression of sympathetic vasoconstrictor peripheral activity [17].

Haloperidol showed a depressive action on pO_2 oscillations only after 2.0 mg/kg, without specificity for any of the structures recorded. The need of a relatively high dose to induce the foregoing changes could be related to the production of vascular modifications by the butyrophenone derivative. In this conjunction, a slight hypotensive action by the drug related to a dual mechanism has been described, namely, a direct action on the smooth vascular fiber and a blockade of catecholaminergic postsynaptic receptors [1, 10, 16].

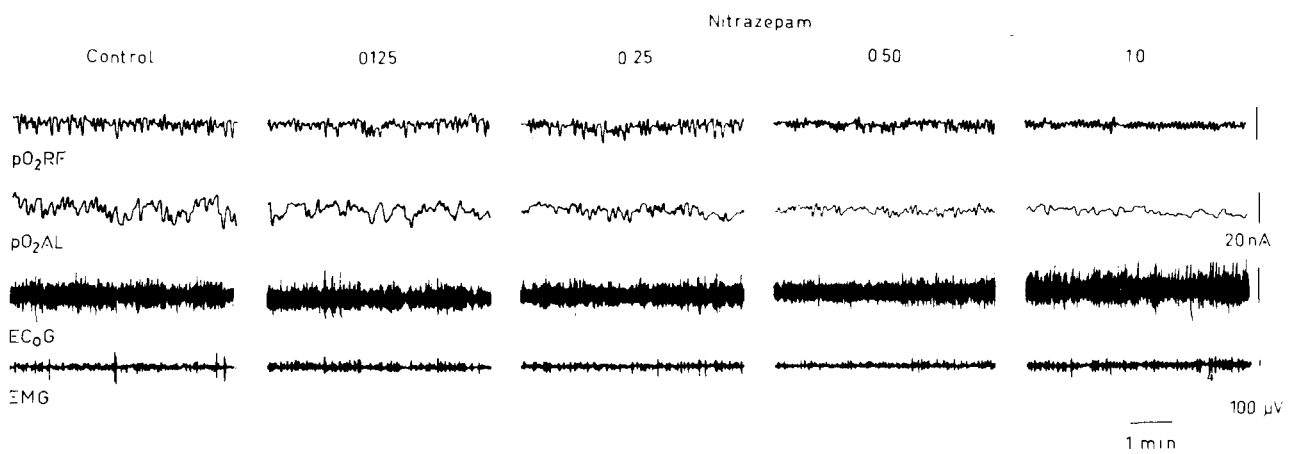

FIG. 3. Dose–response effects of nitrazepam on pO_2 oscillations in the nuclei reticularis pontis caudalis and amygdalae lateralis during slow wave sleep. Abbreviations as in Fig. 1. The numbers on top of the records correspond to mg/kg of drug administered. A dose of 0.25 mg/kg selectively diminished the amplitude of the current waves in AL. After larger amounts of the benzodiazepine derivative the amplitude of oscillations diminished simultaneously in both nuclei in a dose–response related manner.

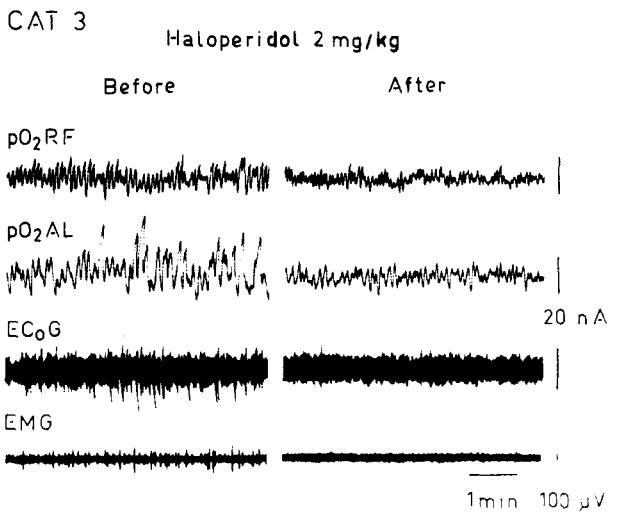

FIG. 4. pO_2 changes in the pontine reticular formation and in the lateral amygdala after haloperidol administration during slow wave sleep. Abbreviations as in Fig. 1. A decrease of the amplitude of pO_2 oscillations in both nuclei is observed 20 min after 2.0 mg/kg haloperidol administration.

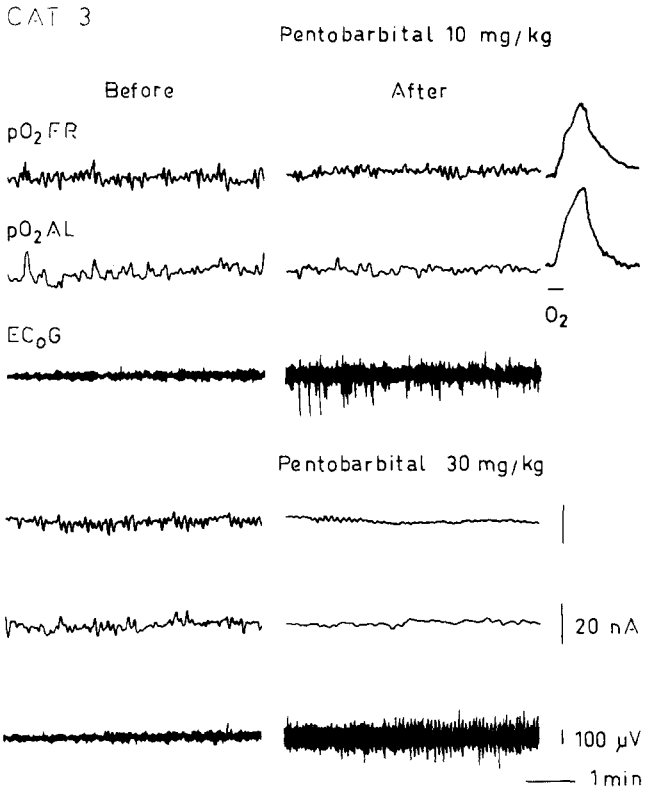

FIG. 5. pO_2 changes in the pontine reticular formation and in the lateral amygdala after pentobarbital administration. Abbreviations as in Fig. 1. The barbiturate provoked a synchronization of the EEG in the previously alert animal. After 10.0 mg/kg a decrease of the amplitude of the oscillations was observed in both structures, while 30.0 mg/kg induced an almost complete disappearance of pO_2 waves. The sudden increase of all pO_2 was still obtained after changing the air of the recording box by pure oxygen (oxygen test).

After sedative doses of pentobarbital, oscillations also showed a decreased amplitude in the lateral amygdala and the pontine reticular formation; anesthetic doses gave rise to an almost total disappearance of pO_2 waves, concurrently with findings by Velluti [21].

These results could be related to the inhibition of oxidative metabolism by pentobarbital [15], although there is no total agreement as to whether this mechanism is responsible for the depression of CNS functions. Sedative or hypnotic doses of the barbiturate do not produce significant cardiovascular effects. Anesthetic doses by i.v. route give rise to a decrease in blood pressure. Nevertheless, it is doubtful whether concentrations of pentobarbital required to produce direct effects on locally controlled brain vessels [19] are obtained after i.p. administration.

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