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Effects of Pentobarbital and Flurazepam on Respiratory Neurons in Undrugged Cats

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DENAVIT-SAUBIÉ, M., A. S. FOUTZ, M.-P. MORIN-SURUN, E. BOUDINOT, J. CHAMPAGNAT AND S. F. GONSALVES. *Effects of pentobarbital and flurazepam on respiratory neurons in undrugged cats.* PHARMACOL BIOCHEM BEHAV 26(4) 647–651, 1987.—Two hypnotic drugs known to enhance GABAergic transmission, a barbiturate (pentobarbital) and a benzodiazepine (flurazepam), were applied locally to respiratory-related neurons (RN) located in the ventral respiratory area in the medulla of non-anaesthetized cats which were either decerebrated or chronically implanted. Pentobarbital applied iontophoretically depressed the spontaneous discharge rate of most RN tested as well as the increase in firing of RN discharge induced by iontophoretic application of glutamate; pentobarbital also potentiated inhibition induced by iontophoresed GABA. Flurazepam applied by iontophoresis or pressure pulses depressed a minority of RN tested and did not enhance GABA-induced inhibition. These results suggest: (1) that inhibition of RN activity through GABAergic mechanisms can be affected by drugs which act at the chloride ionophore but not those acting indirectly through the benzodiazepine binding site, and (2) the effects of the agents on medullary neuronal activity are independent of an effect on the states of consciousness or on structures rostral to the medulla.

Respiration Iontophoresis Pentobarbital Flurazepam Decerebrate cat Chronic cat

EARLIER studies in anaesthetized animals have shown that GABAergic mechanisms are involved in the modulated firing pattern of respiratory neurons [3]. Two drugs which interact with this inhibitory system through different mechanisms were selected for study in non-anaesthetized cats. The barbiturate pentobarbital has been shown to enhance GABAergic neurotransmission by increasing conductance at the Cl^- ionophore; the benzodiazepine flurazepam, on the other hand, acts indirectly through an interaction with the GABA recognition site [8,9]. Iontophoretic application of these agents in decerebrate and chronically implanted animals permitted a study of their local effects on RN independently of indirect effects mediated by other structures involved in the control of breathing, particularly pontine and suprapontine structures and also independently from alterations of the states of consciousness.

METHOD

Decerebrated Animals

This experimental group consisted of 13 cats decerebrated under halothane anaesthesia (Fluotec 3 vaporizer). The trachea was cannulated and catheters were inserted into a femoral artery and a saphenous vein for blood pressure recording and fluid injections respectively. Decerebration was performed at the midcollicular level while both carotid arteries were temporarily clamped. An occipital craniotomy was performed so that electrodes could be lowered into the medullary reticular formation at an angle of 45° in a caudorostral direction. One phrenic nerve was dissected and placed on bipolar silver electrodes to record central respiratory activity. Halothane was then discontinued and the animal paralyzed with gallamine triethiodide (6 mg/kg/hr) and artificially ventilated. End-tidal CO_2 was measured with a Cosma infrared analyzer and was maintained at 4% by adjusting tidal volume on the laboratory built pneumatic respirator. Body temperature was maintained at 38±0.5°C with a CFP model 8142 homeothermic blanket control. Recording began 30 min after both vagus nerves were cut at the midcervical level and 1 to 2 hours after the end of halothane anaesthesia. All data were collected on animals whose arterial blood pressure (measured with a Statham P23Db transducer and a Gould digital meter) never fell below 90 mmHg.

Chronically-Implanted Animals

Eight intact cats were studied in the non-anaesthetized state. Prior to these studies surgical procedures for implanting atraumatic head restraint [6] and for recording sleep-wake patterns, respiratory activity and neuronal activity in the medulla were carried out under pentobarbital anaesthesia (35 mg/kg, IP). Electrodes were placed on the pericruciate and visual cortices for recording the electrocorticogram (ECoG), in the bony orbit for recording the electrooculogram

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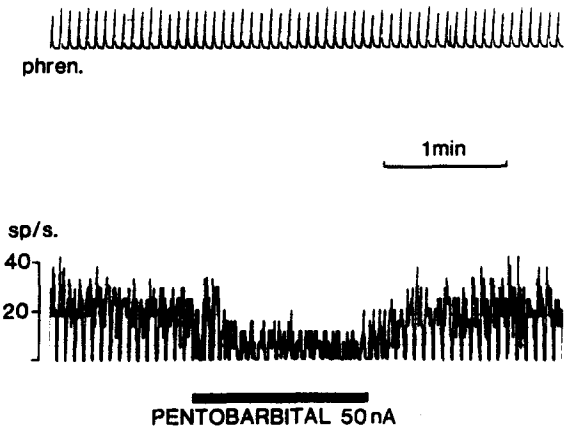

FIG. 1. Effect of pentobarbital on the discharge of a medullary expiratory neuron. Iontophoretic application (50 nA) is indicated by a bar under the tracing. Firing rate decreased by 68% during pentobarbital application. Phren.: integrated activity of the phrenic nerve; sp/s.: ratemeter recording of neuronal spike frequency.

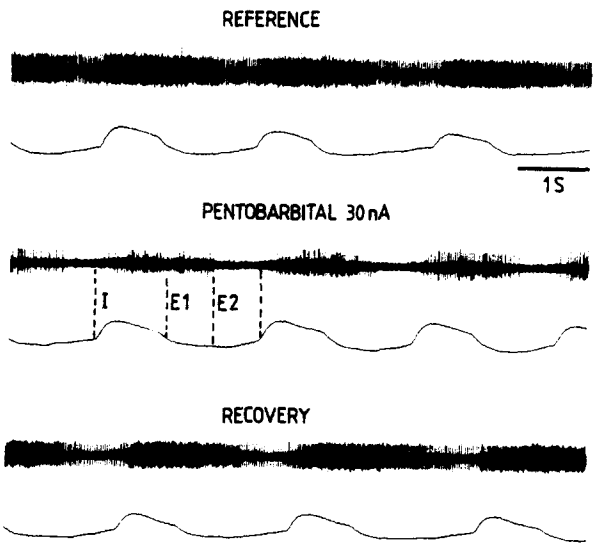

FIG. 2. Effect of pentobarbital ejected with a 30 nA current on the firing pattern of a tonically discharging inspiratory neuron. Upper traces (reference): before drug application. Middle traces: during pentobarbital application 40 sec after the onset of the ejection current. Lower traces (recovery): 1 min after the end of application. I: inspiration; E1: first half of expiration; E2: second half of expiration; respiratory activity was recorded with a thermocouple. Notice the near complete cessation of discharge in E2 during pentobarbital application.

(EOG), and in the sub-occipital muscles for electromyogram (EMG) recording. Respiratory activity was recorded by means of a thermocouple inserted through the nasal passageway with its tip positioned near the nostril opening. All electrodes, the thermocouple and a ground lead were soldered to a 15 pin connector. Three brass bolts inserted in a mound of acrylic cement were later used for head restraint. A Silastic catheter was placed into the right external jugular vein for subsequent injection of drugs. A plastic cylinder was seated in an opening drilled in the bone over the cerebellum so that multibarrelled electrodes could be inserted vertically into the medulla. The dura was removed and antibiotic ointment placed over the cortex. A removable rubber cap was used to seal the opening between the experiments. A stereotaxic reference pole embedded in the acrylic allowed the subsequent positioning of micropipettes.

Each cat was habituated to partial restraint for increasing lengths of time on successive days preceding the beginning of the experiments. For recording the animal was placed in a veterinary restraining pouch and its head was held in a stereotaxic frame by means of the implanted bolts. Eighty two recording sessions (3 to 29 per cat) took place over a period of 3 weeks to 3 months.

Recording Techniques

Extracellular potentials were recorded through a 4 M NaCl filled central barrel of a 7 barrelled micropipette, and amplified with a Grass model P5 preamplifier. The following solutions were used for microiontophoretic application: L-glutamate (1 M, pH 8), γ-aminobutyric acid (GABA 1 M, pH 4.2), sodium pentobarbital (0.2 M, pH 9.2), and flurazepam mono- and dihydrochloride (20 mM and 100 mM, pH 4.5). One channel filled with 165 mM NaCl was used for balancing currents or for passing a current of same intensity and polarity as the drug studied to check for current effects. Laboratory-built iontophoresis equipment was used. Small retaining currents were usually applied to the channels containing active substances to prevent the drugs from diffusing passively out of the micropipette between application intervals. Flurazepam was also applied with pressure pulses delivered by a Neuro Phore PPM-2 pneumatic pump (0.5×10^{-5} to 2×10^{-5} Pa, 2 sec pulses in two cats); the volume of fluid and amount of drug applied were calculated from the diameter of the bubbles measured under a microscope.

In chronic animals, the ECoG, EOG, EMG and respiration were recorded on a 4 channel ECEM polygraph. In decerebrated animals, phrenic nerve activity was integrated using a RC integrating circuit with a leakage resistance. Respiratory activity of both preparations and the frequency of discriminated neuronal discharges counted in 500 msec bins were monitored on an oscilloscope and displayed on a Philips PM 8252 chart recorder. Cell activity, discharge rate, respiration and ECoG were also recorded on magnetic tape (Thorn EMI recorder) and on a Gould model ES 1000 electrostatic printer. Records retained for study occurred during stable sleep-wake states (determined on the basis of the animal's behavior and the polygraph record).

At the end of the acute experiment or on the last recording session in chronic cats, methyl blue was applied iontophoretically to stain the final recording site. Chronic animals were anaesthetized, then perfused with 10% formaldehyde-saline solution. Brain slices 100 μm thick were stained by the Nissl method.

RESULTS

To exclude recordings from fibers, only units excited by iontophoretically applied glutamate were retained for study. A total of 79 RN (57 in chronic and 22 in decerebrated preparations) which responded to glutamate application were recorded long enough to allow their study. All RN were located at the level of the obex in the medullary respiratory area laterally to the nucleus tractus solitarius and extending ventrally to the vicinity of the nucleus ambiguus. Pentobarbital and flurazepam, which are hypnotics when adminis-

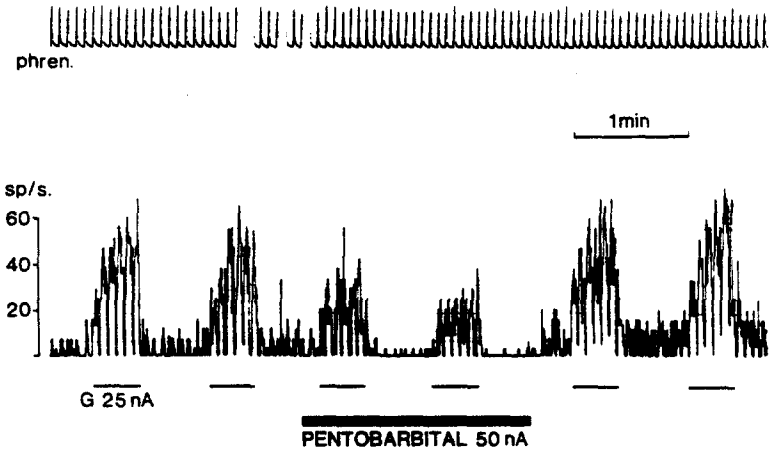

FIG. 3. Effect of pentobarbital (50 nA) on the increased activity of an expiratory neuron induced by glutamate pulses (25 nA, thin horizontal bars). Peak firing rate decreased 40% during pentobarbital application.

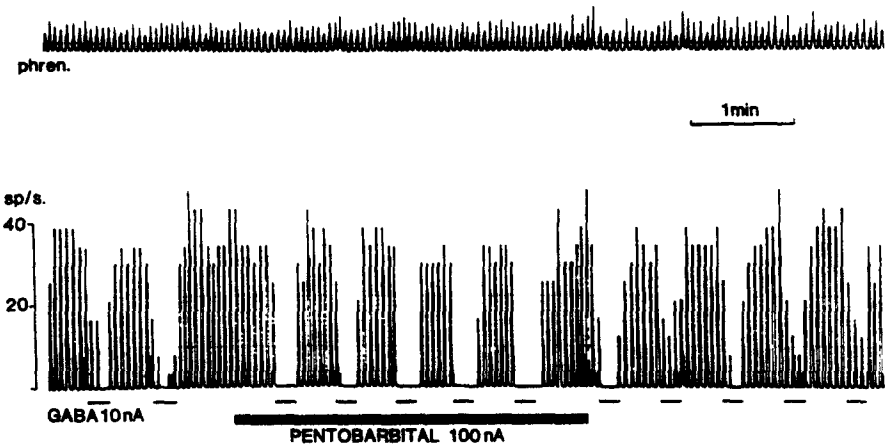

FIG. 4. Effect of pentobarbital (100 nA) on partial inhibitions of an inspiratory neuron induced by short iontophoretic applications of GABA (10 nA). GABA pulses decreased peak firing rate 74% in control conditions and 93% during pentobarbital application.

tered systemically, were tested on cells which exhibited a stable discharge pattern. Respiratory frequency was 19±4 SD breaths/min in decerebrate animals and 30±5 SD breaths/min in the chronic cats. Basal respiratory parameters and blood pressure were never modified by local drug application onto single neurons.

Pentobarbital was iontophoretically applied onto 29 RN (22 in decerebrated and 7 in chronic animals) with ejection currents of 30 to 100 nA. It decreased the discharge rate of 22 RN (mean decrease 66±11% of basal values) and had no effect on 7 RN. Figure 1 shows the effect of pentobarbital on the spontaneous discharge of a phasic expiratory neuron, and Fig. 2 its effect on the discharge of a tonic inspiratory cell. Pentobarbital also: (1) reduced the enhancement in firing rate produced by glutamate iontophoresis (Fig. 3), and (2) potentiated the inhibitory actions of GABA (Fig. 4). A specific effect of pentobarbital during the respiratory cycle is illustrated in Fig. 2. Although the discharge rate decreased in both inspiratory (I) and expiratory (E) phases of the cycle, the decrease was most pronounced during the second half of the E phase, where the firing decreased by 91% relative to a pre-drug reference. Firing decreased by only 46% during the first half of the E phase and by 34% during I phase. This shows that pentobarbital exerted its action on a mechanism which is most active at the end of expiration.

Flurazepam, studied in chronic animals, was iontophoretically applied onto 46 neurons at ejection currents of 60–100 nA. Applied onto 31 RN, flurazepam was active on 13 RN and inactive on 18; when applied onto 15 non modulated cells it was active on 12 cells and inactive on 3 cells. Decreases in discharge frequency were observed in both respiratory and non-respiratory cells. This effect on the firing frequency was usually accompanied by a decrease in spike amplitude. The effects appeared 10–30 sec after the onset of the ejection current, and were often maximal near the end or even just after the end of the application. At that time the spike amplitude was on average decreased to one half of its original value, but in some cases the spike was unaffected and in others it disappeared completely within the background noise. Firing rate for RN which responded to

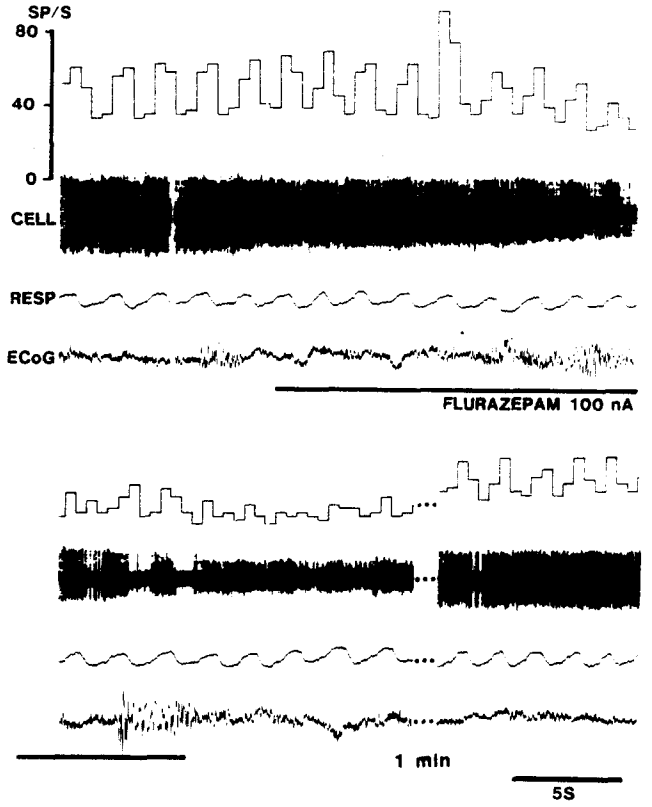

FIG. 5. Effect of flurazepam (100 nA) on a tonically discharging inspiratory neuron. Notice the progressive decrease in spike amplitude and frequency (both decreased by 50%), and the loss of respiratory modulation. The upper and lower traces are in continuity. RESP: respiration, inspiration up; ECoG: electrocorticogram.

flurazepam application decreased by 54±22% SD. Spike amplitude and firing rate recovered over a 3–5 min period, sometimes more. Figure 5 shows a tonically active I neuron on which flurazepam application decreased spike amplitude, firing rate, and respiratory modulation. At the time of maximal effect, the cell discharged with a phasic pattern; this was seen on two other tonically discharging RN.

To rule out the possibility that the effects observed might be current artefacts, the drug was also applied by pressure pulses and gave the same effects as with iontophoretic application. A decrease in spike amplitude and firing rate was obtained. Two RN were depressed with doses of 2 and 0.5 fmoles, and a non modulated cell was also inhibited.

Possible interactions between the effects of GABA and of flurazepam were investigated. When tested in 79 glutamate-sensitive cells (48 RN and 31 non modulated units), GABA invariably decreased or completely inhibited neuronal discharge with ejection currents of 5–50 nA. RN which had a tonic discharge pattern became phasic when GABA was applied. Short GABA pulses (20 sec) inducing a partial inhibition of RN discharge were applied before, during and after flurazepam iontophoresis. Of 22 RN tested, no interaction was seen on 18 cells. On 4 cells GABA-induced inhibition during flurazepam application was enhanced from 48% inhibition to 73%. The overall data appear inconclusive. It seems that for the great majority of RN, any enhancement of iontophoretically applied GABA by flurazepam would be weak at best.

DISCUSSION

Our results show that the depressing effects of pentobarbital on the respiratory function derive from an inhibitory action on RN discharge which appears to involve two distinct mechanisms: a depression of glutamate-mediated excitations and an enhancement of GABAergic inhibitions. This later mechanism may be critical in the effects of pentobarbital on respiration because this drug may affect the modulation of RN as well as their firing rate. This depression of modulation may be due to the fact that inspiratory neurons are submitted to periodic GABAergic inhibitions which are most active in the second part of expiration [3]. The present results have shown that when pentobarbital was applied onto a tonic inspiratory neuron, the most complete depression also occurred during the second half of expiration, when GABAergic input is strongest. Therefore pentobarbital may affect the modulation of RN as well as their firing rate. Direct iontophoretic application of pentobarbital to non respiratory neurons in the medullary reticular formation [13] or to unidentified medullary neurons [8] has been reported to depress most of the cells tested.

Pentobarbital's depression of the spontaneous activity of nonrespiratory neurons was shown to derive from a variety of effects, particularly an enhancement of GABAergic neurotransmission through an action on the Cl^- ionophore and a depression of glutamate-induced excitation [1, 2, 8, 10]. Our results show that these two mechanisms also apply to RN.

Unlike barbiturates, benzodiazepines enhance the gating function of GABA receptors while probably not affecting the properties of the Cl^- channel [8]. In contrast to pentobarbital, flurazepam depressed only a minority of RN tested and failed to enhance GABA-mediated inhibition. Previous autoradiographic studies have shown a low density of benzodiazepine binding sites in the medullary respiratory areas [14,18]. The finding that flurazepam has only weak effects on breathing [7,16] and on RN activity is consistent with that observation. In the few RN which did respond, the high flurazepam currents required to decrease spike frequency also depressed spike amplitude. This suggests an effect of flurazepam on spike generation mechanisms (local anaesthetic action) and complicates any interpretation of flurazepam action on respiratory modulation. High doses of flurazepam have also been reported to produce similar changes in spike amplitude in cerebral cortical neurons [12], brain stem cells [2], spinal cord interneurons and cerebellar Purkinje cells [4,15]. It is noteworthy that opiate agonists applied with high concentrations were also shown to decrease spike amplitude and block spike genesis [5]. It is now generally accepted that flurazepam as well as other benzodiazepines have only minor direct effects on firing rate when administered locally or systemically [4, 11, 17]. These agents act indirectly by enhancing the inhibitory actions of GABA ([11, 12, 17], reviewed in [8]). Our failure to confirm a GABA facilitating action of flurazepam on RN could result in part from the strong modulatory influences exerted on RN. Altering the discharge of RN by a variety of excitatory or inhibitory agents usually required larger ejection currents than for non modulated cells.

In conclusion, pentobarbital and flurazepam, studied independently of their hypnotic properties, both depressed respiratory as well as non-respiratory neurons. However pentobarbital depressed a higher proportion of RN than flurazepam.

ACKNOWLEDGEMENTS

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