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BRIEF COMMUNICATION

Chlordiazepoxide and Morphine Reduce Pressor Response to Brain Stimulation in Awake Rats[1]

MARCUS L. BRANDÃO,*[2] ELISARDO C. VASQUEZ,*
ANTONIO M. CABRAL* AND PIERRE SCHMITT†

Departamento de Ciências Fisiológicas, Centro Biomédico da Universidade Federal do Espírito Santo CP 780, 29000, Vitória, ES, Brasil
†Laboratoire de Neurophysiologie, Centre de Neurochimie du CNRS, 5, rue Blaise Pascal 67084, Strasbourg, France

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BRANDÃO, M. L., E. C. VASQUEZ, A. M. CABRAL AND P. SCHMITT. *Chlordiazepoxide and morphine reduce pressor response to brain stimulation in awake rats.* PHARMACOL BIOCHEM BEHAV 23(6) 1069–1071, 1985.—The effects of intravenous as well as dorsal midbrain injections of morphine and chlordiazepoxide on the blood pressure rise induced by electrical stimulation of the dorsal periaqueductal gray matter (DPAG) were studied in unanesthetized rats. Chlordiazepoxide applied systemically or locally into the DPAG, as well as locally applied but not systemically injected morphine were found to attenuate the centrally-induced hypertension. These data together with others suggest that benzodiazepines as well as local injections of morphine into the DPAG decrease the aversive effect induced by DPAG stimulation.

Dorsal periaqueductal gray matter Aversive brain stimulation Cardiovascular changes
Intracerebral injection Morphine Chlordiazepoxide

ELECTRICAL stimulation of the dorsal periaqueductal gray matter (DPAG) in the rat is known to elicit flight and conditioned escape responses [11,16]. The flight reaction as well as the conditioned escape response induced by DPAG stimulation were found to be attenuated by chlordiazepoxide (CDP) whether injected peripherally [14] or directly into the DPAG [3]. The latency to escape from DPAG stimulation was also increased by microinjection of morphine into the DPAG [8]. These data lead to the suggestion that these treatments decrease the efficiency of DPAG stimulation and therefore attenuate the induced aversive effect.

Electrical stimulation of the DPAG also produces variations in autonomic responses such as an increase in blood pressure, in heart rate and respiration [6]. The present study was aimed at finding out whether CDP or morphine injected either systemically or locally into the DPAG would attenuate, in the awake and unrestrained rat one of the autonomic reaction induced by DPAG stimulation, namely the rise in blood pressure.

METHOD

Male Wistar rats (250–300 g) were used. The detailed surgical procedure was described elsewhere [2]. Briefly, the animals were anesthetized with sodium pentobarbital (45 mg/kg, IP), fixed in a stereotaxic instrument (Labtronics, U.S.A.) and implanted in the dorsal periaqueductal gray with chemitrodes made of a stainless steel guide cannula to which an electrode insulated except at the tip was glued such that its tip came to lie 1 mm below the end of the cannula. The chemitrode was vertically lowered at the lambda, until the tip came to lie 5 mm below the skull surface. The aversive brain stimulation sites used in this experiment were located within or near the DPAG as confirmed by later histological examination.

Seven days later, the rats were anesthetized with ether and the catheter implantation was carried out. To measure the mean blood pressure (MBP), a polyethylene catheter filled with heparinized saline was placed into the abdominal

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[2]Requests for reprints should be addressed to M. L. Brandão, Laboratoire de Neurophysiologie, Centre de Neurochimie du CNRS, 5, rue Blaise Pascal, 67084, Strasbourg, France.

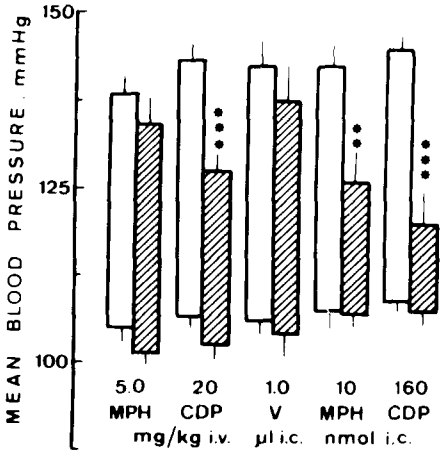

FIG. 1. Effects of intravenously (IV) or intracerebrally (IC) administered chlordiazepoxide (CDP) and morphine (MPH) on mean blood pressure increase elicited by dorsal midbrain electrical stimulation (sine wave, 60 Hz, 15 sec, 17.7–88.4 μA, RMS) in awake and unrestrained rats. V indicates vehicle injection. Horizontal lines at the base of columns represent basal values and columns, responses to brain stimulation before (open columns) or after (dashed columns) treatment. Bars are the SEM. n of each group=6. Asterisks indicate significant differences using Student's paired t test: $^{**}p<0.01$ and $^{***}p<0.001$.

aorta via the femoral artery. Intravenous (IV) injections were performed through another catheter brought into the jugular vein. These catheters were tunneled subcutaneously and exteriorized at the dorsal midcervical region of the neck. The rats were allowed a 24 hr recovery period before the experiments were initiated. In order to record the MBP, the femoral catheter was connected to a polygraph (Hewlett Packard, U.S.A.) *via* a pressure transducer (HP, U.S.A.). The rat was placed into a rectangular chamber (25×20×18 cm). After the animal had carried out the initial period of exploratory behavior, a further period of at least 30 min was allowed before blood pressure was considered to have reached its baseline value.

Brain stimuli were generated by a constant current, sine-wave stimulator. The current intensity was monitored by means of an oscilloscope (Labo, Brazil). Electrical stimuli of 15 sec duration were applied to the DPAG. The intensity was increased until maximum MBP increases of 30–40 mmHg were produced. This stimulus intensity was then kept constant throughout the experiment. Only rats showing these responses with current intensities below 88.4 μA (RMS) were used. The brain stimulation was applied both before and either 45 min after treatment in the case of intravenous morphine or chlordiazepoxide or 30 min after treatment in the case of intracerebral (IC) injection of either drug. Each IV injection was administered in a 1 ml/kg volume and each IC injection was administered in a constant volume of 1 μl during 15 sec using a hand-driven Hamilton 10 μl microsyringe.

Chlordiazepoxide hydrochloride (Psicosedin®, Farmasa, Brazil) and morphine hydrochloride (Farmasil, Brazil) were each dissolved in physiological saline (0.9%), which also served as vehicle control.

Basal MBP was measured immediately before the application of each electrical stimulation. These basal values as well as the maximum changes induced by brain electrical stimulation were averaged. The group means obtained before and after drug or control injections were compared using the paired t test of Student.

RESULTS

As previously reported [15], dorsal midbrain electrical stimulation elicited an immediate increase in the arterial MBP. At stimulation intensities producing a MBP increase of 30–40 mmHG, the animals did not exhibit escape reactions but showed freezing.

As shown in Fig. 1, neither IV nor DPAG injections of CDP or morphine affected basal MBP levels. When injected systemically at the dose of 10 mg/kg, chlordiazepoxide did not significantly affect the increase in MBP induced by DPAG stimulation. This increase was significantly antagonized by chlordiazepoxide injected systemically at the dose of 20 mg/kg or locally at the dose of 160 nmol. The vehicle injected systemically or locally caused no significant effect.

Morphine (10 nmol) applied into the DPAG also reduced the increase in MBP due to electrical brain stimulation. Higher doses of morphine (40 nmol) caused a suppression in only one of 4 tested animals. In the three others, such an injection was followed by a hyperactivity which precluded the recording of MBP. Systemic injection of morphine (2.5, 5 and 10 mg/kg) caused no significant change with regard to the pre-injection level despite the fact that the dose of 10 mg/kg, IV, did already cause slight signs of catatonia. Only data obtained with the dose of 5 mg/kg is shown in Fig. 1.

DISCUSSION

The present results clearly show that the increase in MBP normally induced by DPAG stimulation can be attenuated by chlordiazepoxide injected either systemically or locally into the DPAG. These data are in good agreement with various studies performed in other animal species and showing that the pressor response induced by brain stimulation could be attenuated by systemically injected benzodiazepines [1,2]. More specifically, they are in good agreement with those obtained in the anesthetized rat by Schenberg *et al.* [15] using systemically injected CDP or intracerebral injections of midazolam, another benzodiazepine. However, whereas in the anesthetized rat, a dose of 10 mg/kg of CDP was found to attenuate the increase in MBP [15], such a dose proved insufficient to affect the rise in MBP in the freely moving rat. Interestingly enough, flight behavior induced by an electrical stimulation applied to the DPAG was found to be attenuated when CDP was microinjected into the DPAG at a dose similar to that used in the present study [3]. In addition, intraperitoneal injections of CDP, at even lower doses than those used in the present study, were found to lengthen the latency to interrupt DPAG stimulation (conditioned escape) [14].

Taken together, these data show that benzodiazepines attenuate both a behavioral and an autonomic consequence of DPAG stimulation. It is thus reasonable to assume that these compounds may well attenuate the aversive effects of DPAG stimulation.

A similar conclusion can be drawn as regards morphine if one considers both the reduction of the centrally induced hypertension observed in this study and the increase in the latency to interrupt DPAG stimulation reported by Jenck *et al.* [8] following injection of low doses of morphine into this region. The effects resulting from higher doses of morphine on MBP could not be assessed since such doses induced a

behavioral activation similar to that previously described as "explosive motor behavior" [7].

In contrast to the observations made following DPAG microinjections, systemically injected morphine proved inefficient in attenuating the rise in MBP, even at doses that already induced signs of catatonia. In the unanesthetized dog, morphine was found to differentially affect the behavioral and the autonomic consequences of aversive brain stimulation, the fearlike behavior being attenuated while the cardiovascular consequences remained unaffected [2]. However, the results reported in the literature concerning the action of sytemic injections of morphine on the behavioural responses induced by brain stimulation are at present controversial. Thus, some authors reported an increase in the threshold to interrupt aversive brain stimulation applied to various brain sites [4, 9, 10, 12], whereas some others found no effect with regard to unconditioned [13] or conditioned [14] escape responses induced by DPAG stimulation. Still others [5] reported an increase in the threshold to interrupt subthalamic stimulations, but only in about half of their cats. Given these results, one cannot exclude the possibility that the effects of systemically injected morphine as regards the consequences resulting from aversive brain stimulation may be site specific. It should be added that it is not always easy to clearly assess whether systemically injected morphine indeed attenuates the induced aversive effects or rather produces a mere performance deficit. It therefore matters to simultaneously assess in further studies its effects with regard to both behavioral and autonomic consequences of aversive brain stimulation so as to verify whether or not and in which cases these two kinds of consequences may be differentially affected by the treatment.

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