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BRIEF COMMUNICATION

Cortical Spreading Depression Blocks Naloxone-Induced Escape Behaviour in Morphine Pretreated mice

H. PERÉZ-SAAD[1] AND J. BUREŠ

Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia

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PERÉZ-SAAD, H. AND J. BUREŠ. *Cortical spreading depression blocks naloxone-induced escape behaviour in morphine pretreated mice.* PHARMACOL BIOCHEM BEHAV **18**(1) 145–147, 1983.—Mice treated with morphine (100 mg/kg SC) and 3 hr later with naloxone (30 mg/kg) developed an acute abstinence syndrome characterized by escape attempts (rearing, wall-climbing, jumping) and unconditioned motor (head-shaking, jerking) and visceral signs. Functional decortication by spreading depression (SD), elicited 15 min before naloxone injection by epidural application of 25% KCl, abolished the escape behaviour without interfering with other abstinence signs. Electrophysiological recording confirmed reliable generation of cortical SD waves under the conditions of the experiment. The SD effect indicates that the escape behaviour of morphine dependent mice is a conditioned compensatory response to the unconditioned effects of the drugs.

Morphine dependence Naloxone Abstinence signs Escape behaviour Jumping Spreading depression

THE morphine abstinence syndrome elicited in morphine-tolerant animals by abrupt suppression of the morphine effect by an antagonist [6, 8, 11] is characterized by a wide spectrum of somatic and vegetative symptoms probably mediated by various neurotransmitter systems [3, 4, 5, 10]. The abstinence signs can be divided into two main categories: (a) Unconditioned motor and viseral reactions due to the changes elicited in the lower brain structures by morphine withdrawal. (b) Complex motor reactions integrated in higher brain structures, representing attempts of the animal to counteract the discomfort produced by the unconditioned symptoms. The aim of the present study is to differentiate these two categories of abstinence signs by the functional decortication technique [1].

BEHAVIOURAL EXPERIMENTS

Method

Male albino mice of the JCR strain (30 g body weight) were used throughout. They were housed in group cages and had free access to food and water. One week before the actual experiment, the animals were prepared for elicitation of cortical spreading depression (CSD) [1,7]. Under pentobarbital anesthesia (40 mg·kg^{-1}), the skull was exposed and periost removed. A thin layer of dental acrylic was spread over the clean surface of the occipital, parietal and frontal bones and removed after it became hard, as a 1 mm wide plate. Trephine openings 3 mm in diameter were made in the parietal bones over both hemispheres. Special care was taken to leave the dura intact. The acrylic plate was replaced and covered with the skin. The borders of the wound were sutured over the acrylic plate but a space was left between them to facilitate a non-painful reopening of the wound.

Three groups of 10 animals were used in the behavioral experiments: unoperated, operated and experimental. All groups were injected with morphine (100 mg/kg SC). A few minutes later, the scalp suture was cut and the acrylic plate was removed. A filter paper soaked in 0.9% NaCl solution (operated controls) or in 25% KCl solution (experimental groups) was applied to the trephine openings two hours and forty minutes after the injection of morphine. In these groups the running behaviour induced by morphine was automatically counted during two 15-min periods before and after application of the filter paper. For this purpose the animals were placed into a circular runway (5 cm wide, 120 cm circumference), two opposite sections of which were equipped with pairs of floor electrodes. The mouse short-circuited the electrodes when passing through this section of the runway and the current pulse (1 μA) was used to activate a solid state circuit which counted all transitions between sections. After conclusion of the running test, i.e., 3 hours after injection of morphine and 20 min after KCl application, the animals were injected with naloxone (30 mg·kg^{-1}) [6,11] and placed into a transparent plastic cylinder (35 cm high and 15 cm in diame-

[1]Visiting scientist from the National Center for Scientific Investigations, Havana, Cuba.

[2]Requests for reprints should be addressed to Dr. J. Bureš, Institute of Physiology, Czechoslovak Academy of Sciences, Vídeňská 1083, Prague 4—Krč, Czechoslovakia.

ter). They were observed in 20 sec periods separated by 40 sec intervals, during 20 min. The presence of the abstinence signs in each 20 sec period of observation was noted.

Results

The injection of morphine produced in all mice the typical Straub tail sign and running behaviour. In the control groups these signs were present in the entire 3 hour period preceding the injection of naloxone and were not affected by epidural application of NaCl. In the experimental group the running behaviour was completely abolished after the epidural application of 25% KCl. The circular runway counts for the two 15-min periods were 100%/99%, 100%/98% and 100%/0% in unoperated controls, operated controls and experimental animals, respectively.

In the control groups, the abstinence syndrome was mainly expressed by escape behaviour, with the following characteristics: (a) Tendency of the animals to assume a sitting posture and to look upward to the rim of the cylinder. (b) Changes from the sitting to the standing position with the forelimbs touching the walls of the cylinder. (c) Rapid forelimb shaking (FS) during transition from the sitting to the standing position. (d) Attempts of the animals to climb the walls of the cylinder. (e) Jumping. For the quantification purposes, the sitting behaviour and FS was classified as one group (Forelimb Shaking) and the standing and climbing behaviours as another group (Standing). In addition to the above escape behaviours, head shaking and jerking were also observed but their incidence was low. The vegetative abstinence signs were expressed by urination, defecation and increased respiratory frequency. Figure 1 shows the percentage of animals displaying the above signs in the control and experimental groups: while manifestations of escape behaviour were completely abolished by SD, other abstinence symptoms were not affected or even increased. The total number of samples with jumps was lower in operated than in unoperated controls, $t(18)=1.7$, $p>0.05$, but the difference was not statistically significant. On the other hand, incidence of samples with standing was increased, $t(18)=2.42$, $p<0.05$. Grooming behaviour, not present in the control groups, appeared in 80% animals during SD.

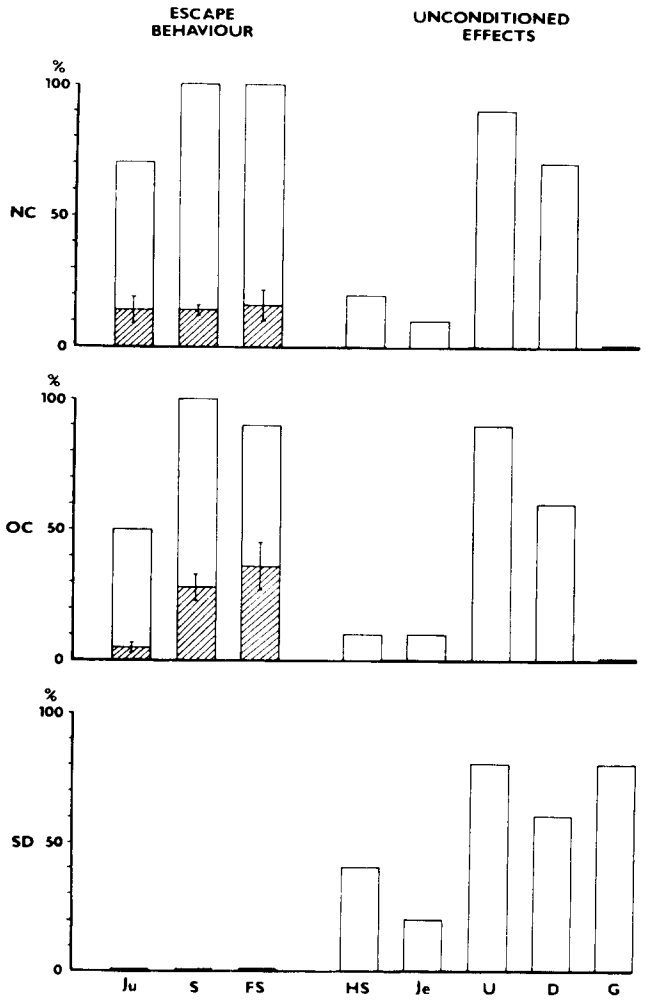

FIG. 1. Incidence of various abstinence signs in morphine pretreated unoperated controls (NC), operated controls (OC) and functionally decorticated rats (SD) during 40 min after naloxone injection. The columns indicate percentage of rats displaying the particular sign. The shaded part of the columns indicate the percentage of samples in which the sign was present. The vertical bars denote SEM values. D—defecation, FS—forelimb-shaking, G—grooming, HS—head-shaking, Je—jerking, Ju—jumping, S—standing, U—urination.

ELECTROPHYSIOLOGICAL EXPERIMENTS

Method

Six additional animals were prepared as the operated controls. A week later, they were anesthetized with pentobarbital (30 mg·kg^{-1} IP), the sutures were cut and the acrylic plate removed. A trephine opening 2 mm in diameter was made in the right frontal bone 1 mm rostral and 2 mm lateral from bregma. The mouse was fixed in the stereotaxic apparatus and a glass capillary (1–2 μm tip, 2–3 MΩ) filled with physiological saline was stereotaxically introduced into the caudate nucleus. The fluid in the capillary electrode was connected through a saline bridge to a calomel cell electrode. Wicks of two other calomel cell electrodes were placed one on the exposed neck muscles (reference), the other on the cortex exposed by the frontal opening. The electrodes were connected to a two-channel polygraph recorder. After the baseline was established, a filter paper soaked with 25% KCl was placed onto the right parietal opening and DC potential changes were recorded during 40 min.

Results

Figure 2 shows a typical recording of slow potential changes accompanying the SD waves in the cortex and in the caudate nucleus following the application of 25% KCl onto the cerebral cortex. In 50% of the animals the first CSD wave penetrated into the caudate nucleus. Subsequent CSD waves generated at 4 to 6 min for at least one hour were observed only in the cortex. Occasionally, they appeared at long irregular intervals also in the caudate nucleus.

DISCUSSION

The electrophysiological experiments indicate that SD can be induced in mice one week after the surgical preparation of the animals. Up to this time, dura protected by the acrylic plate remained in good condition. The cortical effects of KCl and low incidence of SD waves penetrating into the caudate nucleus were the same as reported in similar SD experiments in rats [1,2]. Furthermore, the application of KCl in unanesthetized animals produced typical motor signs of CSD (disappearance of placing reactions).

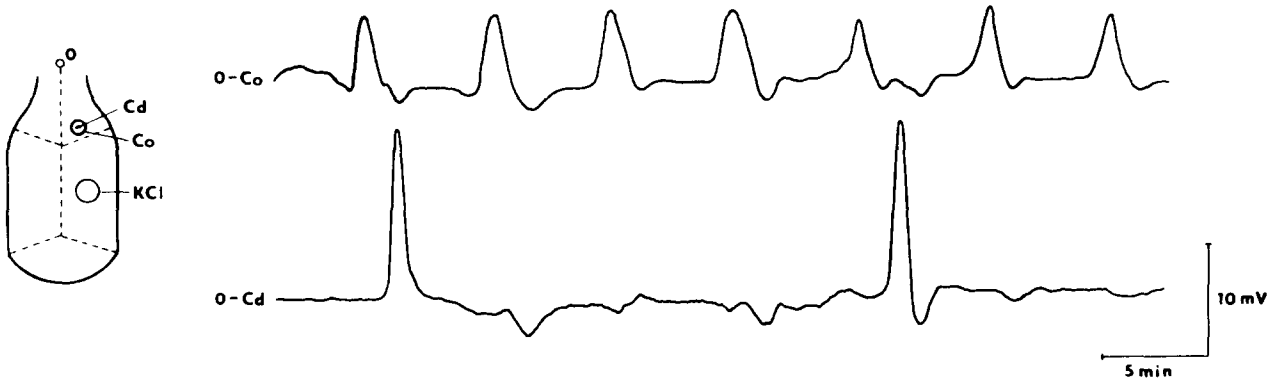

FIG. 2. Cortical and caudate spreading depression elicited by application of a filter paper soaked with 25% KCl onto the exposed cortical cortex of an anesthetized mouse. The brain scheme shows the position of the recording electrodes and of the KCl application site. O–1 and O–2: slow potential recording from the cortex and from the head of the caudate nucleus. Calibration: 10 mV, 5 min.

The behavioural experiments show that CSD produces complete abolition of the naloxone induced escape behaviour in morphine pretreated animals. As expected the vegetative signs of the abstinence syndrome were not affected. Although these results can be due to remote subcortical effects of CSD [1] it is more likely that functional decortication blocks the cortically mediated components of the abstinence syndrome.

The corticalisation of the escape behaviour of morphine dependent mice suggests that jumping is a conditioned compensatory response to the unconditioned reactions elicited by the drugs [9]. This suggestion is supported by other observations: (a) The animals can orient the jumps in a definite direction: upward when they are placed into a high cylinder or forward when put on an elevated platform. (b) The preparative phase for the jump is clearly expressed and is more pronounced after the failure of the first escape attempts. (c) The incidence of jumps varies considerably between animals subjected to the same treatment probably in accordance with the previous individual experience of the animals with jumping. The inexperienced animals adopt sitting or standing posture and look toward the top of the cylinder or try to escape by climbing the wall.

The forelimb shaking behaviour deserves a special comment. This motor sign regularly appeared when a sitting animal raised his forelimbs in an attempt to stand up. FS always stopped when the animal resumed the sitting posture. The short duration of the FS (about 1 sec) indicates that the animal tries to stop the uncontrollable shaking of the forelimbs by inhibiting the eliciting activity. In this respect FS resembles the intentional tremor observed in patients with cerebellar lesions performing a discrete aimed movement. SD interferes with this rapid, repetitive involuntary movement, probably integrated in the lower brain structures, indirectly, by impairing spontaneously emitted operant behaviour in general.

The secondary, mediated nature of the jumping behaviour decreases the usefulness of this sign for quantification of physical dependence and for establishing the neurotransmitter systems involved in the primary effect of morphine. Abstinence signs not affected by functional decortication seem to be better suited for this purpose.

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