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BRIEF COMMUNICATION

Abolition of Conditioned Heart-Rate Responses in Rabbits Following Central Administration of [N-MePhe^3, D-Pro^4] Morphiceptin[1]

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LAVOND, D. G., M. D. MAUK, J. MADDEN, IV, J. D. BARCHAS AND R. F. THOMPSON. *Abolition of conditioned heart-rate responses in rabbits following central administration of [N-MePhe^3, D-Pro^4] morphiceptin.* PHARMACOL BIOCHEM BEHAV **19**(2) 379–382, 1983.—Rabbits were initially habituated to a tone and then give 15–20 paired trials where the tone was followed by periorbital shock resulting in conditioned heart-rate decelerations to tone onset and an acceleration of heart-rate following shock offset. The animals were matched for learning performance and divided into two groups. Each animal received a microinfusion into the region of the fourth ventricle of either the opiate [N-MePhe^3, D-Pro^4] morphiceptin, a highly selective mu receptor agonist, or a mixture of the morphiceptin analogue and the opiate antagonist naltrexone. Administration of the morphiceptin analogue eliminated the conditioned bradycardia to the tone but not the acceleration to the shock. Rabbits given the mixture continued to show conditioned heart-rate decelerations. Previous studies have shown that opiates abolish a recently learned conditioned nictitating membrane response. These effects are consistent with the hypothesis that the opiate effect on conditioning is due to an attenuation of conditioned fear.

Conditioned fear Opiates Heart-rate conditioning

IN RECENT studies we have shown that morphine and certain opioid peptides cause selective and naloxone-reversible abolition of the recently learned, classically-conditioned nictitating membrane (NM)/eyelid response in the rabbit [12,13]. The opiates have no effect at all on the unconditioned reflex response (UCR), have no effect on conditioned stimulus (CS) evoked activity in the primary auditory system, and abolish the conditioned response (CR) on the trial immediately following injection and before the next unconditioned stimulus (UCS) is given. Thus, it would seem that the opiates may be acting directly on some neuronal substrate of the associative process. This effect is due entirely to action within the central nervous system and can be obtained with substances that are highly specific for the mu receptor (e.g., morphiceptin and its analogues). Several sites of central administration were examined, including the lateral ventricles, third ventricle and the fourth ventricle. The most immediate and reliable effects were obtained with administration in the rostral portion of the fourth ventricle, suggesting primary action on structures rich in mu opiate receptors in the vicinity of the periaqueductal/periventricular region.

Theoretical behavioral analyses of aversive learning have emphasized two processes: an initial conditioned central state, e.g., conditioned emotional response (CER) or conditioned fear, and subsequent learning of discrete motor responses adapted to the situation [9, 18, 19, 21, 22]. In a variety of situations where conditioned fear modulates behavior, opiates have been shown to produce selective effects on CRs (e.g., conditioned emotional response [17], startle potentiation [4], and escape-avoidance [6]). The (mostly autonomic) responses associated with conditioned central

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states develop rapidly [24,29] and have been shown to fade as the animal masters discrete, striated muscle CRs [20,24]. Interestingly, we have recently shown that the effects of morphine on classically conditioned NM/eyelid responses fade as the animals become overtrained [11].

The above considerations have led us to suggest that there may be considerable physiological support for a two-process approach to aversive learning. We have hypothesized that the first process of conditioned fear is essential for the subsequent learning of the discrete motor response (see [9]), that the two processes have different neuronal substrates, at least in part, and that the differential opiate action on conditioned NM/eyelid responses is mediated through an action on conditioned fear process [27,28].

Classically conditioned cardiovascular responses to an aversive UCS are commonly viewed as an index of conditioned fear [8,24]. In the rabbit the reflex response to a periorbital shock UCS includes marked tachycardia but the conditioned response is bradycardia, a substantial slowing of the heart-rate [8, 20, 24]. If the centrally-mediated, opiate abolition of the recently learned NM/eyelid response is a result of action on a component of conditioned fear, then we would also expect central administration of opiates to abolish the classically conditioned heart-rate slowing response in the rabbit.

METHOD

Adult New Zealand White rabbits (N=19) were first operated upon in order to implant chronic, indwelling cannulae into the fourth ventricle, using Halothane for anesthesia. The coordinates were AP +13 mm, ML 0 mm, and between 10 and 11 mm DV with respect to bregma [14]. For each rabbit the dura was incised 2 to 4 mm lateral to the midline so that the cannula could be inserted and moved medially in order to deflect the sagittal sinuses from the cannula path. The cannula was then lowered until cerebrospinal fluid could be withdrawn from the anterior fourth ventricle. The cannula was cemented in place with dental acrylic. The rabbit was then allowed 7 to 10 days to recover from the surgery.

Each rabbit was adapted in a restrainer in the conditioning chamber for 1 hour the day before training. At this time two small loops of stainless steel wire were sutured subcutaneously and periorbitally to the left eye. The rabbit was also shaved on his chest, on his back on the right side, and on the right ear. Small folds of skin were held in plastic earclips at each of these sites to simulate placement of the heart-rate recording electrodes.

On the next day the recording electrodes were reattached and each rabbit was first adapted to the tone (CS, 1 kHz, 85 dB, 5 sec, 60 sec ITI) for 15 CS-alone trials in order to habituate unconditioned cardiac deceleration. In order to give the experimenters immediate feedback, heart-rate was evaluated by dividing the rate immediately before CS offset by the baseline rate immediately before CS onset and multiplying by 100 (i.e., a percentage of baseline rate). A rabbit was considered to be adapted if the percentage was 99% or greater in a block of 5 trials. This criterion was chosen on the basis of pilot animals and recent literature (see [5]). Statistical analyses later where made upon the actual frequencies of heart-rate. Heart-rate itself was recorded with Ag-AgCl surface electrodes, and determined by measuring the distance taken by 10 heartbeats (polygraph speed at 50 mm per sec) and converting this to an average frequency.

After adaptation, at least 15 trials were given in which the 5 second tone (CS) was followed by 500 milliseconds of faceshock (UCS, 2 mA, 60 Hz). Criterion for learning was a deceleration of heart-rate before CS offset that was at or below 95% of the rate before CS onset in a block of trials. This criterion was based upon pilot animals and reports in recent literature (e.g., see [5]). Of 19 rabbits, 7 failed to reach this criterion within 30 or 40 trials and were eliminated from the study. This protocol was chosen because it has been reported that rabbits usually learn cardiac deceleration within 5 to 10 trials and that repeated training of rabbits causes eventual acceleration of heart-rate following CS onset, probably due to increased somatic activity [23, 24, 25]. Two additional animals were rejected because of technical difficulties. The remaining 10 animals were assigned to one of two groups on the basis of matched percentage performance on the last block of paired training. This matching procedure also matched the two groups in terms of total number of paired trials (15, 15, 15, 20 and 25 trials for the control group, 15, 15, 15, 20 and 30 trials for the experimental group).

These remaining 10 rabbits were administered either 12 nmol of [N-MePhe^3, D-Pro^4] morphiceptin, a long-lasting, potent and highly specific mu receptor agonist [1,2], or a mixture of the morphiceptin analogue with 10 nmol of the opiate antagonist naltrexone hydrochloride (N=5 in each group) into the fourth ventricle. All compounds were dissolved in Ringer's solution immediately prior to infusion. The injections were administered in a 20 μl volume delivered over 100 seconds. Training trials continued within 30 seconds after the completion of the infusion. Each rabbit was given a total of 15 paired trials under his respective drug condition.

RESULTS AND DISCUSSION

Following histological confirmation of the cannulae locations, the rabbits' performances were statistically evaluated by using the actual heart-rate frequencies (using 10 heartbeat intervals) during the PreCS and PreUCS periods. This was done in order to evaluate independently the effects of the experiment on baseline (PreCS) and active (PreUCS) heart-rates. Thus, postexperiment statistics were based upon the actual frequencies, and not the percentages used for experimenter feedback at the time of training. These frequencies were based upon the 10 heart-beats before CS onset, the 10 heart-beats before UCS onset, and the 10 heart-beats after UCS onset, each averaged for a block of 5 trials for each rabbit, because we hypothesized an intertrial effect and not an intratrial difference between groups.

Heart-rate during adaptation, paired training and drug conditions is plotted in Fig. 1 as the percentage difference between PreUCS heart-rate and PreCS heart-rates. Both groups adapted to the CS-alone condition and learned to decelerate (a positive score in the figure) their heart-rates in anticipation of UCS onset. The group administered the morphiceptin analogue in conjunction with naltrexone continued to show conditioned cardiac deceleration, which decreased as training continued. These facts are consistent with the findings of previous investigators (e.g., [8,24]). In contrast, the conditioned heart-rate slowing response was immediately abolished in those rabbits administered the opiate agonist alone, evaluated with a two-way mixed ANOVA for pre- and postinjection blocks within subjects, $F(1,10)=8.3$, $p<0.05$.

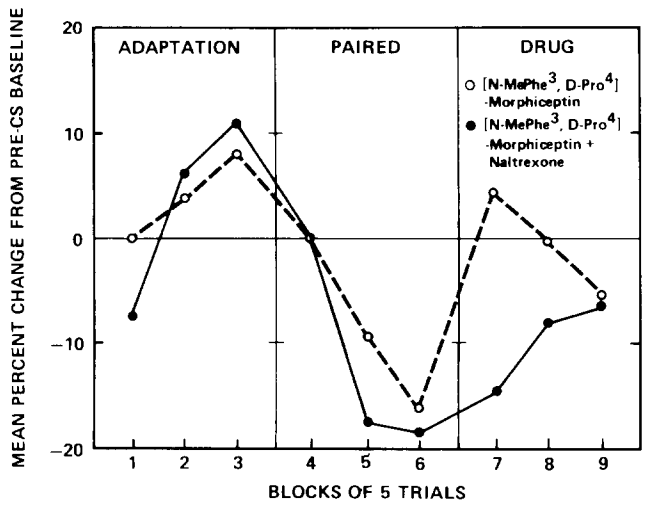

FIG. 1. Mean percentage differences of heart-rate for two groups (opiate alone: open circle, opiate/naltrexone: closed circle) over the course of adaptation, paired training and drug conditions. Positive percentage scores (100%×(PreUCS-PreCS)/PreCS) reflect heart-rate acceleration. Since individual animals were given additional paired training as needed, only the final three blocks (5 trials each) were considered.

Similar analysis of baseline heart-rate revealed a significant treatment effect, $F(5,40)=11.6$, $p<0.001$, which introduces the possibility that the opiate effect on conditioned heart-rate responses is produced by a floor effect or other factors related to a change in baseline heart-rate. However, that the effects on baseline for both the opiate alone and opiate/antagonist groups were indistinguishable ($F<1$) argues strongly against this position. The treatments × block interaction revealed no reliable differences, $F(5,40)=1.1$, $p>0.05$, indicating the effect on baseline heart-rate was similar for both groups. An additional post-hoc, matched t-test revealed no difference between the baseline for the two groups following injection, $t(8)=0.67$, $p>0.05$. Further, correlational analysis of the effect on the CR and the effect on baseline heart-rate revealed small correlations in the opposite direction expected by the position that the effect on the CR was related to a change in baseline (opiate-alone $r=-.36$, opiate/naltrexone group $r=-.46$ and combined $r=.04$, $p>0.05$ for each). Thus, despite a reliable effect on baseline heart-rate, the fact that this change occurred equally after treatments that abolish the CR and those that had no effect on the CR argues strongly that the effect on the CR is not related to the change in baseline. Similarly, others have shown that heart-rate CRs are unaffected by large variations in baseline [10].

It is unlikely that the opiate effect on heart-rate CRs is a result of an altered "perception" of the aversive UCS. There was no effect of either treatment on the unconditioned tachycardia response evoked by the UCS, matched $t(8)=0.48$, $p>0.05$. Moreover, the conditioned heart-rate response reattained habituation levels of responding in 3 of the 5 rabbits (88, 93, 100, 101 and 103% heart-rate) during the first tone presentation, before shock, on the first trial following drug administration.

In sum, fourth ventricular application of a low dose of [N-MePhe³, D-Pro⁴] morphiceptin, a highly specific mu agonist, causes abolition of the conditioned heart-rate slowing response in the rabbit. The effect is blocked by concurrent administration of naltrexone. The opiate has no effect on the reflex heart-rate response to the shock UCS.

This same opiate administration causes selective abolition of the discrete, adaptive learned NM/eyelid response if it has just been learned [13]. We suggest that the selective opiate abolition of both types of learned responses might be due to a common action on some part of the "conditioned fear" system localized in the brain stem ventricular area.

In other studies we have shown that the lateral, ipsilateral cerebellum is essential for learning, memory and relearning of the NM/eyelid response, regardless of the degree of training or overtraining, suggesting that the cerebellar system may serve to code the memory trace for the discrete, adaptive learned response [15,16]. Consistent with this interpretation is the fact that overtraining protects against the effects of opiates on the NM/eyelid CR [12]. It is as though the presumed cerebellar system develops some degree of "functional autonomy" when the specific adaptive response is well-learned; conditioned fear is no longer so critical.

There are, of course, other essential neuronal substrates for the learned heart-rate response, including portions of the amygdala and the hypothalamus [3, 8, 26]. Interestingly, administration of opiates to the central nucleus of the amygdala attenuates acquisition of the conditioned heart-rate slowing response in the rabbit [5] but has no effect on the just learned NM/eyelid response [12].

We suggest that there may be localized and possibly common opiate actions on some part of the conditioned fear circuitry in the vicinity of the fourth ventricle necessary for both responses—learned heart-rate and the initial learning of the NM/eyelid response. These suggestions are obviously quite speculative. However, they are amenable to experimental test and are consistent with the large literature implicating opioids in learned fear and anxiety [7,17] and with the behavioral literature on aversive conditioning [9, 18, 19, 21, 22].

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