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Pituitary and Brain β-Endorphin in Male and Female Rats: Effects of Shock and Cues Associated With Shock[1]

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FARABOLLINI, F., R. P. W. HEINSBROEK, F. FACCHINETTI AND N. E. VAN DE POLL. *Pituitary and brain β-endorphin in male and female rats: Effects of shock and cues associated with shock.* PHARMACOL BIOCHEM BEHAV **38**(4) 795–799, 1991.—The present experiment was designed to study whether or not prior exposure to inescapable shock is accompanied by sex-dependent changes in pituitary and central levels of immunoreactive β-endorphin, which is proposed to play an important role in opioid analgesia induced by aversive stimulation. Further, the effects of brief reexposure (5 min) to the chamber where inescapable shock was experienced earlier, were established in both sexes. Elevated levels of β-endorphin were found 24 hours after inescapable shock, in the anterior pituitary of males and in the midbrain periaqueductal gray of both males and females. Reexposure to the experimental chamber only affected β-endorphin levels if shock had been experienced in this chamber. Reexposure after inescapable shock reduced β-endorphin content of the arcuate nucleus of males and β-endorphin content of the periaqueductal gray of males and females. The present results are related to previous findings of sensitization and conditioning of analgesic reactions. The sex differences found in the present experiment are discussed with respect to sex-dependent behavioral consequences of inescapable shock.

Aversive stimuli β-Endorphin Analgesia Behavioral deficit Male and female rats

IN male rats, exposure to inescapable unavoidable electric shocks (IS) has often been found to produce subsequent poor escape performance in a shuttle-box shock-escape task. This escape deficit was minimized when shocks during preexposure were escapable. The IS-induced deficit in escape performance was, therefore, explained by assuming that IS will promote learning act-outcome independence, which will interfere with subsequent learning (13). This associative deficit proposed by the "learned helplessness" hypothesis was suggested to be accompanied by a deficit in response initiation resulting from a reduced incentive to attempt to escape (motivational deficit). As a consequence, reduced activity is observed in the presence of shock which is incompatible with escape responding typically requiring large amounts of activity (14). Pain modulating mechanisms have been implicated in the activity deficit induced by IS. A transient reduction in pain perception (analgesia) was observed immediately after both escapable and inescapable shock, but only the latter was mediated by endogenous opioid systems. Furthermore, at longer intervals after IS, but not after escapable shock, analgesia could rapidly be reinstated by brief reexposure to a few shocks. Increased response latencies in a shock-escape task after IS may, therefore, be a consequence of a reinstatable, opioid mediated reduction in pain perception (14,15).

Strong evidence implicates opioid mechanisms in the activity deficit induced by prior experience with IS (12, 14, 15). Of the various endogenous opioids, β-endorphin appears to be among the most relevant ones for analgesia. The peptide produces strong as well as prolonged analgesia, whereas reduced pain perception induced by aversive stimulation is accompanied by changes in peripheral and central levels of β-endorphin (1). β-Endorphin is derived from pro-opiomelanocortin (POMC), which also contains the full sequence for adrenocorticotropin hormone (ACTH). POMC is present in the anterior and intermediate lobes of the pituitary and is found in the brain in the arcuate nucleus (ARC) of the hypothalamus and in the nucleus tractus solitarius (6). POMC neurons from the ARC project to midbrain and brainstem areas which have been associated with analgesia. Both electrical stimulation and microinjection of opiates and opiate antagonists at

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these sites were effective in depressing or modulating pain perception. Important projection areas of POMC ARC neurons involved in antinociception include the paraventricular nucleus of the thalamus (PVN) and the periaqueductal gray (PAG). Futhermore, β-endorphin released from the anterior pituitary (PIT) into the plasma appears to be involved in at least some forms of analgesia (24).

Analgesia at a 24-hour interval after IS required a few shocks to be reinstated, therefore, it was proposed that exposure to IS sensitized opioid mechanisms (12,15). This sensitized state may be reflected in altered opioid levels in relevant areas and it was the purpose of the present experiment to examine this possibility. Furthermore, conditioning of an analgesic response to environmental cues associated with IS has been described. Conditioned analgesia was proposed to contribute to the deleterious effects of IS on escape performance (3). The present experiment also explored the effects of brief reexposure to environmental cues associated with IS on opioid concentrations.

Recent findings have shown that male and female rats differ with respect to the behavioral consequences of IS. Performance of a shuttle-box shock-escape task was disrupted after IS treatment in males and in females but the deleterious effects of IS were much larger in males than in females (8,22). Pain modulating mechanisms have been implicated in sex-dependent behavioral consequences of IS: sex differences may have resulted from a stronger analgesic response in males than in females (22). This suggestion is supported by findings that analgesia induced by aversive events and of both nonopioid and opioid nature as well as analgesia induced by systemic administration of opioids is smaller in females than in males (4).

In a previous experiment from our laboratory, male and female rats were exposed to IS and 24 hours later were either sacrificed immediately or after a brief (5 min) reexposure to the shock chamber. Neurochemical changes induced by these manipulations were studied in this experiment (Heinsbroek et al., submitted). The present paper presents data of immunoreactive β-endorphin measured in various parts of the brain and PIT of these same animals.

METHOD

Subjects

Forty female and 40 male Wistar rats were obtained from Animal Supply House, TNO (Zeist, The Netherlands). At arrival the animals were 9 weeks old, they were group-housed in standard cages (single-sex) and maintained under a reversed light-dark cycle (lights on from 3:30 p.m. to 3:30 a.m.). Three weeks after arrival at the laboratory the experiments were started. Prior to experimentation, subjects were weighed twice a week in the experimental room. In the animal quarters food and water were always available to the subjects.

Apparatus

The experiments took place in two Grason-Stadler (Model 1111-L) rodent operant conditioning chambers. The floor (30 × 30 cm), consisting of 23 stainless steel grids, spaced 1.25 cm apart, was connected to a shock-generator (Grason-Stadler Model 700). Two levers, normally located 10 cm above the floor on both sides of a pellet retrieval unit, were removed. A white houselight was present in an upper corner of the intelligence panel. The test chambers were enclosed in a sound-attenuated compartment (Grason-Stadler Model 1101) with a fan to provide fresh air. The programming of the experimental conditions was accomplished using Grason-Stadler 1200-series of programming equipment, located in the experimental room itself.

Procedure

All subjects were placed one at a time in one of the conditioning chambers. Half of the group was put in a chamber where 60 scrambled footshocks (1 mA, 2-s duration) were presented on a variable time interval of 30 s (VT 30). The other half was confined for a similar period of time in the other chamber where shocks were never given. Following this session of about 30 minutes subjects were replaced in their home cage for an interval of 24 hours. After this interval one half of each group of subjects was sacrificed by rapid decapitation. The remaining subjects were briefly (5 min) reexposed to the chamber before being sacrificed. The design of the experiment resulted in 4 groups of males and 4 groups of females ($n = 10$ per group). For both sexes one group was shocked and reexposed, one group was shocked and not reexposed, one group was not shocked and reexposed and one group was not shocked and not reexposed.

After sacrifice brains were quickly removed from the skull. Brain material obtained from these same animals (frontal cortex) was also used to measure neurotransmitter activity (Heinsbroek et al., submitted). After rapid dissection of the frontal cortex, the remainder of the brain was put on a cooled (−3°C) stainless steel platform, ventral surface up. Three transversal cuts made at the level of the hypothalamus produced 2 sections of about 3 mm thickness. The cuts roughly corresponded with the following stereotaxic coordinates, −2.2, −5.2 and −8.3 from bregma (19). Appropriate areas were punched out with stainless steel cannulas of different diameter. From the rostral section (−2.2 to −5.2) the paraventricular nucleus of the thalamus (PVN) was sampled with a cannula of 1.0 mm diameter. A 2.0 mm cannula was used to sample part of the ventral hypothalamus from the rostral section, including the arcuate nucleus (ARC). The periaqueductal gray (PAG) was taken from the caudal section (−5.2 to −8.3) by using a 1.6 mm cannula. The anterior pituitary (PIT) was removed from the base of the skull and separated from the neurointermediate lobe.

Brain samples were directly put into 0.5 ml of hot 0.1 N acetic acid and placed in boiling water for 5 min. All samples were subsequently frozen at −80°C and stored until assayed. Prior to radioimmunoassay samples were homogenized and centrifuged. β-Endorphin was assayed in the supernatant following the methods described in detail elsewhere (18).

RESULTS

Levels of β-endorphin as determined in the 3 brain areas and the anterior pituitary (PIT) are depicted for the 8 experimental groups in Figs. 1 to 4. These data were analysed by using a 3-way analysis of variance (ANOVA) with sex, shock and reexposure as main factors. Newman-Keuls ($\alpha = 0.05$) tests were used for making post hoc comparisons among groups.

PIT

Figure 1 shows the data for the PIT. IS increased β-endorphin concentrations in the PIT, but this effect depended on the sex of the animal, $F(1,72) = 4.30$, $p < 0.05$ (sex × shock). In males IS resulted in a significant increase in β-endorphin in the PIT and this was not observed in females. A significant effect of the main factor sex was also observed, $F(1,72) = 13.08$, $p < 0.001$, primarily resulting from the high β-endorphin concentrations encountered in males exposed to IS. Reexposure after prior experience with IS resulted in a 21% reduction of β-endorphin in the PIT of

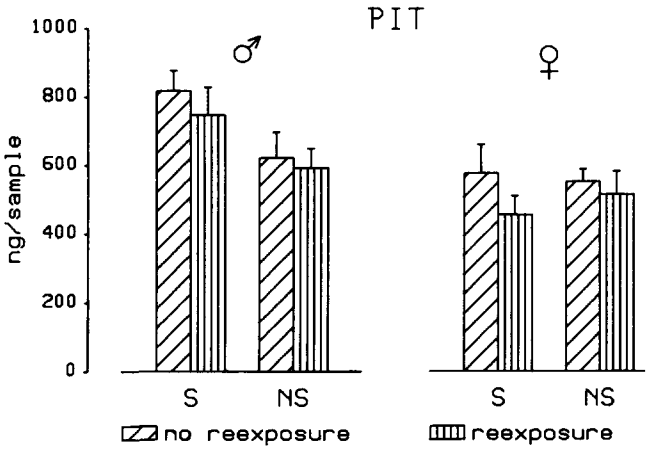

FIG. 1. Mean amount of β-endorphin (+s.e.m.) in the anterior pituitary (PIT) of male and female rats. Subjects had been exposed to inescapable shock (S) or had been confined to the test chamber without shock (NS). After a 24-hour interval subjects were sacrificed immediately, or were reexposed for 5 min to the test chamber prior to sacrifice.

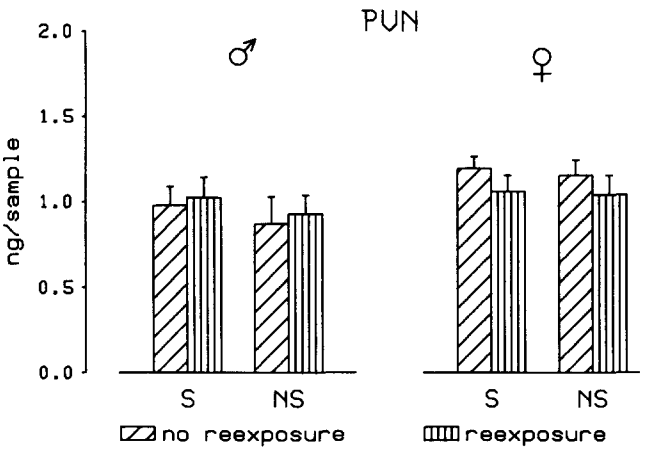

FIG. 3. Mean amount of β-endorphin (+s.e.m.) in the paraventricular nucleus of the thalamus (PVN) in male and female rats. Subjects had been exposed to inescapable shock (S) or had been confined to the test chamber without shock (NS). After a 24-hour interval subjects were sacrificed immediately, or were reexposed for 5 min to the test chamber prior to sacrifice.

females, whereas a 9% reduction induced by reexposure after IS was found in males. These changes were not found to be significant.

ARC

Figure 2 presents the data for the ARC sample. ANOVA revealed a significant main effect of shock, $F(1,72)=8.99$, $p<0.01$, a significant interaction between sex and shock, $F(1,72)=4.45$, $p<0.05$, and a significant interaction between sex, shock and reexposure, $F(1,72)=6.00$, $p<0.05$. Post hoc comparisons showed that reexposure after previous experience with IS reduced β-endorphin levels in the ARC of males. In females no differences were found.

PVN

In Fig. 3 results for the PVN sample are depicted. Only a significant effect of sex was found after ANOVA, $F(1,72)=4.24$, $p<0.05$. Overall, β-endorphin concentrations in this area were higher in females as compared to males.

PAG

β-Endorphin levels in the PAG are presented in Fig. 4. Over-

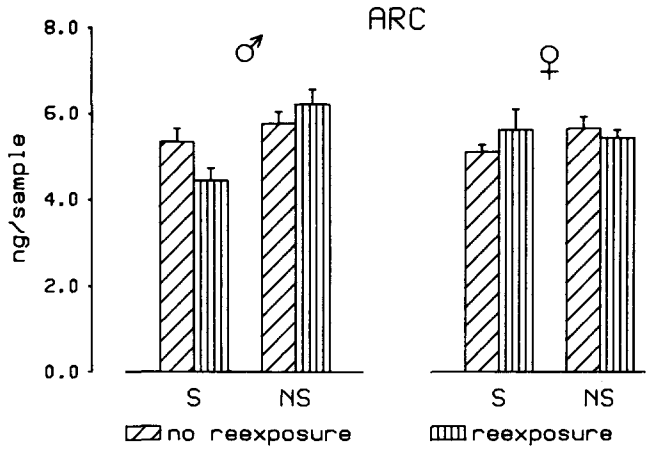

FIG. 2. Mean amount of β-endorphin (+s.e.m.) in part of the ventral hypothalamus including the arcuate nucleus (ARC) of male and female rats. Subjects had been exposed to inescapable shock (S) or had been confined to the test chamber without shock (NS). After a 24-hour interval subjects were sacrificed immediately, or were reexposed for 5 min to the test chamber prior to sacrifice.

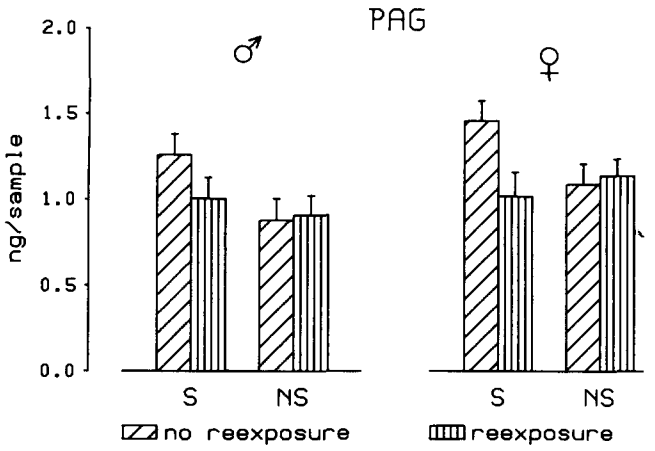

FIG. 4. Mean amount of β-endorphin (+s.e.m.) in the periaqueductal gray (PAG) of male and female rats. Subjects had been exposed to inescapable shock (S) or had been confined to the test chamber without shock (NS). After a 24-hour interval subjects were sacrificed immediately, or were reexposed for 5 min to the test chamber prior to sacrifice.

all, β-endorphin levels in the PAG were slightly higher in females as compared to males, $F(1,72)=3.89$, $p=0.05$. After prior exposure to IS, increased β-endorphin concentrations were measured in this area, main effect of shock: $F(1,72)=4.49$, $p<0.05$. However, increased β-endorphin concentrations were only found in groups exposed to IS and not reexposed to the shock chamber. Reexposure after IS resulted in a rapid reduction of β-endorphin in the PAG, shock and reexposure interacted significantly, $F(1,72)=5.13$, $p<0.05$. Post hoc tests confirmed these findings by revealing a significantly higher β-endorphin concentration in groups only exposed to IS as compared to all other groups. Effects of shock and reexposure on β-endorphin in the PAG were not sex-dependent.

DISCUSSION

In the present experiment immunoreactive β-endorphin levels in the PIT and in various parts of the brain were affected by prior experience with IS and by reexposure to stimuli associated with IS. In addition, some of the effects of the experimental manipulations were found to be sex-dependent. The β-endorphin content of the PIT was increased in males 24 hours after IS and this was not observed in females. Similarly, β-endorphin in the PAG was elevated by IS 24 hours earlier and the elevation was observed in males and females. The PAG and the ARC revealed a selective reduction in the β-endorphin content in response to reexposure, after prior experience with IS. In the ARC this reduction was only noted in males, whereas in the PAG, β-endorphin was decreased in both males and females in response to reexposure.

One of the intriguing findings of the present experiment is the considerable elevation of β-endorphin in the PIT of males 24 hours after IS. This increment may have resulted from elevated synthesis of PIT β-endorphin as alterations in synthesis of β-endorphin after aversive stimulation have been reported. In rats chronically exposed to aversive stimulation, an increase in mRNA coding for POMC was found in the PIT and this was associated with elevated β-endorphin content of the PIT (1,5). In addition, biosynthesis of β-endorphin may also be accelerated by other mechanisms. In particular, acute stimulation was found to result in both a more rapid production of POMC, without alterations in mRNA, and an accelerated conversion of POMC to its products (1). What particular mechanism is responsible for the presently observed elevation is unclear at present. It can, however, be speculated that the induction of mechanisms involved in synthesis will alter the reactivity of the system. As an example, chronic stimulation was not only found to substantially increase β-endorphin content of the PIT, but also resulted in a more rapid release (1). The IS-induced elevated level of β-endorphin in the PIT, found in the present experiment, may, therefore, reflect a "sensitized state." Twenty-four hours after IS, β-endorphin may be produced and released more rapidly from the PIT in response to a new challenge.

Increments in plasma β-endorphin as a result of aversive stimulation have been found to be paralleled by reduced PIT content of β-endorphin (9, 16, 20, 23). In the present experiment, 5-min reexposure did not result in a significant reduction of β-endorphin levels in the PIT, although a trend in this direction was noted. Akil et al. (1) proposed that increased biosynthesis as a result of aversive stimulation could mask a change in PIT content of β-endorphin following stimulation. As already proposed, the elevated β-endorphin levels after IS could reflect alterations in synthesis and this may, in turn, have prevented observation of a reduction in β-endorphin in response to reexposure. It is of particular interest to note that the elevation of PIT β-endorphin after IS was clearly present in males and absent in females. Moreover, a decrease in response to reexposure was more prominent in females than in males. These findings suggest that IS increased synthetic mechanisms in the PIT of males and not of females.

PIT β-endorphin may be involved in the reinstatable, opioid analgesia, observed after IS and proposed to mediate the activity deficit (15). Maier and his collaborators (14,15) suggested that exposure to IS will temporarily sensitize opioid mechanisms, resulting in a rapid analgesic response to subsequent challenges. Perhaps the increment in synthetic processes, implicated by findings of the present experiment, subserves this sensitized state. If true, this mechanism also appears to provide a substrate for sex differences in the behavioral effects of IS. Twenty-four hours after IS male rats were slower to escape from shock than females and this may have involved a more stronger analgesic response in males than in females (22). Perhaps exposure to IS sensitizes opioid mechanisms through an induction of synthetic activity in the PIT of males, but not of females. It must, however, be emphasized that this interpretation of the present findings is speculative, in particular as the role of PIT β-endorphin in the IS-induced escape deficit is uncertain. Several findings indicate that PIT β-endorphin is involved in analgesia induced by aversive stimulation. Substantial elevations of β-endorphin in plasma, released from the PIT, were found in response to 5-min footshock (16,20) or 5-min cold swim (23). These changes in plasma β-endorphin were associated with profound analgesia (16,23). Furthermore, treatments that prevent or block PIT output of β-endorphin (hypophysectomy, dexamethasone) interfered with some forms of analgesia (24). On the other hand, these treatments were not found to block the escape deficit effects of IS, whereas they did block the analgesic consequences of IS (10). In addition, although IS-induced analgesia and IS-induced deficit in escape performance were both blocked by the opioid antagonist naltrexone, these effects were not found when the peripherally acting quarternary naltrexone was used (25).

Central β-endorphin levels were also found to be affected by the present experimental manipulations. Levels of β-endorphin in the ARC were reduced in response to reexposure after IS in males but not in females. This finding may be of interest in view of the fact that the ARC contains POMC neurons with extensive projections to parts of the brain which are critically involved in antinociception. Electrical stimulation and opiate microinjection in midbrain POMC projection areas produced profound analgesia which was partly blocked by opiate antagonists (1,15). However, analgesia was not observed following stimulation of the ARC (1). Therefore, the functional significance of the sex-dependent reduction in β-endorphin in the ARC remains obscure.

Alterations in β-endorphin content of the PVN were not noted in the present experiment. On the other hand, changes in the PAG were very conspicuous. In both males and females, previous experience with IS resulted in elevated β-endorphin content of the PAG and reexposure after IS rapidly reduced the opioid peptide content of this area. Similar to the changes in PIT β-endorphin, increments in PAG content 24 hours after IS, are likely to result from increased biosynthesis. In contrast to the PIT, β-endorphin levels in the PAG were responsive to brief reexposure. The reduction in central levels of β-endorphin was specifically found after experience with IS, suggesting that a conditioned response to environmental cues associated with IS was involved. In the literature, unconditioned aversive stimulation has been reported to affect central levels of β-endorphin (2, 11, 16, 21, 23). Of particular interest are the observations that β-endorphin levels in

diencephalon and mesencephalon periventricular tissue were reduced after footshock (16,20). Moreover, electrical stimulation of the midbrain PAG elicited a reduction in β-endorphin levels in the PAG accompanied by an analgesic response, suggesting that release of β-endorphin from opiate terminals in this area underlies hypoalgesia (17). Pavlovian conditioning of an analgesic response, normally provoked by unconditioned aversive stimuli, has been reported in the literature and has been claimed to be mediated by opioid mechanisms (24). Previously, Kinscheck et al. (7) obtained data showing that conditioned analgesia in rats is attenuated by lesioning areas of the PAG. Therefore, the conditioned response of PAG β-endorphin observed in the present experiment may be of relevance for conditioned analgesia.

Previous experiments showed that IS more severely retarded subsequent escape performance of males than of females, and this may have resulted from a stronger analgesic response in males than in females (22). The present findings show sex differences in β-endorphin responses to IS and to reexposure in some (PIT, ARC) but not in other (PAG) parts of the endogenous opioid system. The relevance of these biochemical changes for sex differences in behavioral responses to aversive stimuli remains to be investigated in future experiments.

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