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Original article

Attenuation of learned helplessness in rats after transplant of adrenal medulla into the spinal cord

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Summary – The influence of an adrenal medullary transplant into the lumbar subarachnoid space on learned helplessness, an animal model of depression, was examined. The transplanted rats were found to be less susceptible than sham-operated animals to become helpless after administration of inescapable shocks. The effect was attributed to release of opioid peptides by chromaffin cells as it was reversed by naloxone. The viability of the transplanted tissue was verified by electron microscopy.

adrenal medullary transplant / opioid peptides / naloxone / learned helplessness / depression / rat

INTRODUCTION

Over recent years our laboratory has studied the involvement of the endogenous opioid system in the learned helplessness paradigm in the rat. This animal model of depression has features in common with the human condition and exhibits a similar response to drug treatment (Willner, 1984, 1990). It has also been increasingly used for investigating the psychobiology of depression. In previous studies we showed that the endogenous opioid system was involved in this model of depression, as administration of either enkephalins or inhibitors of enkephalin metabolism, which increases endogenous opioid levels, enhanced the escape and avoidance responses in rats previously exposed to inescapable shocks (Tejedor-Real et al, 1993, 1995).

It is now well known that chromaffin cells from the adrenal medulla produce significant amounts of catecholamines and other pharmacologically active neuropeptides, such as the opioid peptides derived from the proenkephalin precursor (Yang et al, 1980; Livett et al, 1981; Willson et al, 1982). In addition, Sagen et al (1986a, b; Sagen and Wang,1990a) demonstrated that sensitivity to pain is reduced after transplantation of either solid rat adrenal medullary tissue or bovine chromaffin cells into the subarachnoid space of the rat spinal cord. This analgesic effect has been attributed in part to release of opioid peptides from the transplanted cells since it is antagonized by naloxone.

The present study was designed to determine whether adrenal medullary implants into the subarachnoid space reduced the susceptibility of rats to learned helplessness. In particular, we wished to reveal the possible involvement of the opioid system in this model of depression using, in part, a non pharmacological procedure.

MATERIALS AND METHODS

This study was performed on 40 male Wistar rats (weighing 200–250 g at the beginning of the experiments). It was conducted according to the guidelines laid down in the *Guide for Care and Use of Laboratory Animals* (1978) of the Institute of Laboratory Animal Resources National Research Council.

Surgery

The animals were anesthetized by intraperitoneal administration of ketamine hydrochloride (Ketolarr, Parke Davis, 0.25 mL/rat) and diazepam (Valium10r, Roche, 0.1 mL/rat). A microsurgical lumbar laminectomy was

carried out and a small slit was opened in the dorsal side of the dura mater. The left adrenal gland was carefully removed and quickly placed in a vessel filled with oxygenized Ringer lactate solution, where the medulla was dissected out under a stereoscopic magnifying viewer. The adrenal medulla was then introduced into the subarachnoid space and slid among the roots of the cauda equina. In the sham-implanted animals a small piece of muscle was used instead. Animals exhibiting motor abnormalities following surgery were eliminated from the study.

Training and testing

The procedures for helplessness training and testing have been described elsewhere (Tejedor-Real et al, 1993, 1995). Briefly, 6 weeks following tissue transplantation animals were subjected to 60 scrambled, randomized inescapable electric footshocks (1 mA, 15 s duration, intershock interval 5–80 s). In order to evaluate escape and avoidance performance, avoidance training was carried out 48 h later in a shuttle-box (30 avoidance trials: 3 s light and 3 s shock; 30 s between trials). Avoidance sessions were performed for three consecutive days in the morning, and the number of escape failures was recorded; an escape failure was when the rat failed to cross into the other compartment during shock delivery.

Behavioral deficits were expressed as the mean number of escape failures + SEM. The results were compared using both Kruskal-Wallis and Mann Whitney U tests, and considered significant if $P < 0.05$.

Drugs

Half the implanted and sham-implanted animals received naloxone (2 mg/Kg) subcutaneously 15 min before the escape and avoidance test. The rest of the animals received vehicle injections.

Histology

In order to determine the underlying morphological changes, 2 months after transplantation and following the testing procedures, the animals were perfused via the aorta with buffered mixed aldehydes, and the spinal cord regions containing the implants were dissected out. Some graft tissues were processed for dissecting microscopic examination, while others were processed for electron microscopy.

RESULTS

Histology

The transplanted tissue was readily identifiable under a dissecting microscope. The transplants appeared healthy and were seen to contain numerous chromaffin cells on sections stained with hematoxylin and eosin (fig 1). Ultrastructural examination revealed that the grafted animals harbored viable clusters of medullary cell groups in the subarachnoid space. Chromaffin cells in the graft contained numerous granules of noradrenaline (fig 2).

Fig 1. Light micrograph showing the grafted chromaffin tissue 2 months after surgical insertion of adrenal medullary tissue into the subarachnoid space of the spinal cord. A cluster of chromaffin cells can be seen in the subarachnoid space of the spinal cord. The arrows indicate the edge of the implant. Hematoxylin and eosin stain (× 100).

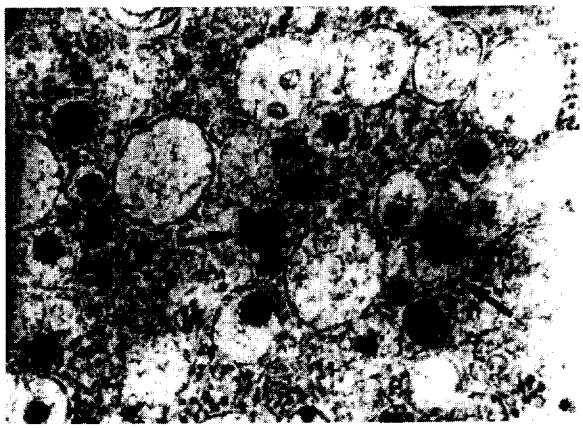

Fig 2. Electron micrograph of a section cut through a portion of adrenal medullary tissue implanted 2 months earlier in the subarachnoid space. The chromaffin cells are readily identified by their large granules (× 50,000) (arrows).

Shuttle-box test

In the shuttle-box test (escape failure number), Kruskal-Wallis analysis revealed a significant dif-

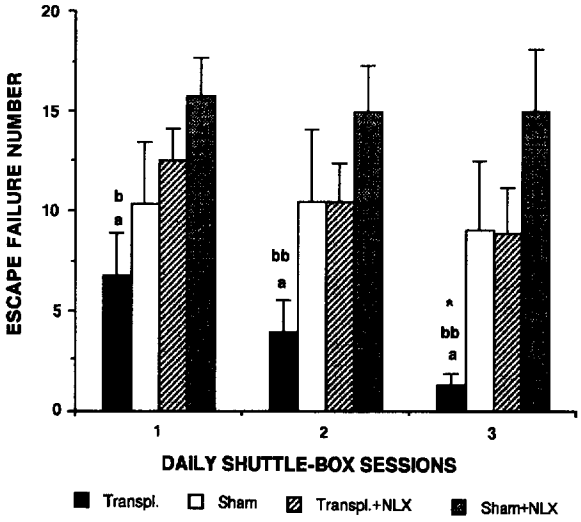

Fig 3. Mean number of escape failures during the 30 trials of the three daily shuttle-box sessions in sham, transplanted, transplanted + naloxone (NLX) and sham + NLX rats after inescapable shock pretreatment. The bars represent the SEM. * $P < 0.05$ vs sham animals; a: $P < 0.05$ vs transplanted + NLX; b: $P < 0.05$ vs sham + NLX; bb: $P < 0.01$ vs sham + NLX (Mann Whitney U-test).

ference between groups during the three shuttle-box sessions (1st session: H = 8.72 [*df*3] $P < 0.03$; 2nd session: H = 9.08 [*df*3] $P < 0.02$; 3rd session: H = 10.65 [*df*3] $P < 0.01$). Posthoc comparisons showed that the implanted animals preexposed to inescapable shocks exhibited fewer escape failures than did the control animals by the first session and became statistically significant by the third shuttle-box session (3rd session: U = 13 [8–9], $P < 0.05$). During the three shuttle-box sessions, fewer escape failures were also noted compared to the naloxone-treated animals, both implanted (1st session: U = 20.5 [9–10], $P < 0.05$; 2nd session: U = 15.5 [9–10], $P < 0.05$; 3rd session: U = 14.6 [8–10], $P < 0.05$) and sham-implanted [1st session: U = 11.5 [9–9], $P < 0.05$; 2nd session: U = 8.5 [9–9], $P < 0.01$; 3rd session: U = 4 [8–8], $P < 0,01$) (fig 3). Naloxone effectively antagonized the effect of the implant, since the scores of the animals treated with this drug before the escape and avoidance test were comparable to those of the sham animals. The highest number of escape failures was observed in the sham-implanted animals treated with naloxone.

DISCUSSION

The results of the present study demonstrated that an adrenal medullary transplant into the spinal cord of rats led to a decrease in the number of escape failures in the learned helplessness paradigm. The results also indicated that learned helplessness was prevented rather than reversed by this treatment.

In addition to producing catecholamines, chromaffin cells produce high levels of pharmacologically active neuropeptides including opioid peptides, neuropeptide Y, vasoactive intestinal polypeptide, neurotensin, somatostatin, cholecystokinin and calcitonin (Livett et al, 1981; Kuramoto et al, 1987; Gaumann and Yaksh, 1988; Bommer and Herz, 1989; Ortega and Sagen, 1993). Any or all of these agents may have participated in the diminished acquisition of helplessness induced by an adrenal medullary transplant into the spinal subarachnoid space. The involvement of the catecholaminergic system in this model of depression has been reported by numerous authors (Anisman et al, 1980; Petty and Sherman 1980; Sherman and Petty, 1982; Martin et al, 1986 a, b), while others have suggested a role for neuropeptide Y (Widerlöv et al, 1991; Tejedor-Real et al, 1994).

In preliminary studies we showed that the opiate antagonist naloxone blocks the effect of inhibitors of enkephalin metabolism, which are able to activate the endogenous opioidergic system (Tejedor-Real et al, 1993), and the effect of the tricyclic antidepressant imipramine in this model of depression (Tejedor-Real et al, 1995). These results could suggest, in line with earlier observations (Devoize et al, 1984; Martin et al, 1986; Isemberg and Cicero, 1984), that there might exist an opioid component in the antidepressant action of tricyclics. This would be in accordance with the theory of the implication of opioids in depression (Extein et al, 1982; Naber, 1993).

To assess whether opioid release could be responsible for the reduction in learned helplessness after the adrenal medullary transplant, we treated the implanted animals with naloxone. Naloxone was found to abolish the effect of the transplant, indicating that it was mediated, at least in part, by release of opioid peptides. In this respect, Sagen and Kemler (1989) reported two-fold higher basal levels of met-enkephalin-like immunoreactivity in the spinal cord superfusates of animals with adrenal medullary transplants than in those of sham-operated controls. Because naloxone by itself increases helpless behavior, it could be suggested that a tonic effect of the opioid system is necessary to maintain escape performance; thus, tricyclic antidepressants or enkephalin catabolism inhibitors or adrenal medullary transplant, which enhances opioid levels, would interact with the escape deficits induced in rats.

Nevertheless, naloxone is able to antagonize the effect of several nonopiod drugs (Sawynock et al, 1979). Some of these latter effects may be due to these agents interacting with endogenous opiate systems in a manner which has yet to be elucidated or they may reflect actions unrelated to opioid-receptor blockers.

Transplantation of adrenal medullary chromaffin cells into the spinal cord subarachnoid space has been shown to reduce pain sensitivity (Sagen and Kemmler, 1989; Ruz-Franzi and González-Darder, 1991; Sagen et al, 1991; Vaquero et al, 1991) which is thought to be mediated by the release of opioid peptides from the implanted cells, since it is also blocked by naloxone.

The antidepressive activity of adrenal medullary implants in other tissues has also been investigated. For example, Sagen et al (1990) found that implantation of catecholamine-containing medullary tissue into the frontal cortex also prevented the development of learned helplessness.

The present results suggest that behavioral changes can be induced by transplantation of adrenal medullary tissue into the spinal cord. The concurrent activation of both catecholaminergic and opioid systems in the spinal cord appears to be synergistic since chronic treatment with amitriptyline, an antidepressant that inhibits the reuptake of noradrenaline, enhances the action of opiate-like material in the spinal cord and potentiates morphine analgesia (Hamon et al, 1987). Moreover, we have shown that amitryptiline enhances the analgesia induced by adrenal medullary transplant into the spinal cord (Ortega-Alvaro et al, 1994).

Nevertheless, these preliminary results raise a number of questions which will need to be addressed in future studies. Do the behavioral changes stem from diffusion of neuroendocrine substances released from the grafted cells into relevant regions of the central nervous system? In this sense, Lindberg and Yang (1984) found that the ratio of high molecular weight enkephalin-containing peptides to pentapeptides is much greater in the adrenomedullary tissue than in the brain; processing within the adrenal medulla is apparently less complete than in the brain. It is possible that early cleavages occur more rapidly than later cleavages, thus resulting in a pool of intermediate-sized enkephalins which are only slowly cleaved further to pentapeptides. These last steps might take place far away from the site of implantation.

In conclusion, the results of the present study demonstrated that an adrenal medullary transplant into the spinal cord subarachnoid space protects rats from learned helplessness after treatment with inescapable shocks. The effect was attributed, at least in part, to the release of opioids from the implanted tissue. Implanted chromaffin tissue may thus provide a long-lasting source of enkephalins in addition to other neuroactive substances.

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