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The Effect of Opiate Treatment on the Postdecapitation Reflex and Monoamine Metabolism in the Rat Spinal Cord

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PLAZNIK, A., M. G. DE SIMONI AND S. ALGERI. *The effect of opiate treatment on the postdecapitation reflex and monoamine metabolism in the rat spinal cord.* PHARMACOL BIOCHEM BEHAV 19(3) 427–429, 1983.—Postdecapitation seizures (PDR) are a spinal reflex which seems regulated by some monoaminergic neurons present in the spinal cord (S.C.). In order to better characterize the role of dopaminergic neurons in PDR, we studied the effect of treatment with opiates, which are known to increase dopamine (DA) and serotonin (5HT) metabolism in the brain, on the duration of PDR and on the metabolism of DA and 5HT in S.C. Morphine, given either IP or ICV, reduced the duration of PDR and increased DA metabolism. Both effects were more evident after systemic administration. [D-Ala^2]Met^5 enkephalin amide acted similarly to ICV administered morphine. Biochemical and behavioral effects were significantly correlated.

Spinal cord Dopamine Dopamine metabolism Postdecapitation reflex

KNOWLEDGE of monoaminergic systems operating in the spinal cord (S.C.) is still scanty. Somewhat more is known about the distribution and function of noradrenaline (NE). Noradrenergic fibres innervating the S.C. originate mostly from NE cells in the medulla oblongata and pons [10,11]. It is postulated that spinal NE plays an important role in mediating supraspinal control mechanisms over spinal reflexes [17]. The "postdecapitation seizure" or postdecapitation reflex (PDR) is an example of a reflex mostly dependent on the integrity of the spinal NE system. Selective depletion of spinal NE or pharmacological blockade of postsynaptic adrenergic receptors inhibits it [13, 16, 19, 21].

However it was shown recently that serotonin (5HT) also contributes, either directly or by interaction with other monoaminergic systems, to the mechanisms controlling the PDR [16,17]. It was also reported that dopamine (DA) exists in the rat spinal cord independently from NE neurons and that it is likely to be present in the terminals of descending axons [5,11]. Some pharmacological manipulations and neurotoxic lesions have in fact demonstrated the independent functioning of DA/NE systems in the rat spinal cord [5].

The role of DA in PDR is less clear. Some authors claim no DA involvement in this reflex [14]. However, Pappas *et al.* [17] found that selective depletion of brain DA could significantly inhibit the PDR. Although applied treatment did not affect NE levels in the brain and S.C., it strongly reduced the duration and vigor of the PDR. It is well known that opiate drugs increase the turnover of central DA as a consequence of a positive feed-back resulting from a fall in the release of this transmitter [1, 2, 7, 12].

The present study had three objectives: (1) to establish the effect of these drugs on S.C. dopaminergic system; (2) to further characterize independent functions of the DA system in the S.C.; (3) to relate any such effects to the PDR.

METHOD

Male CD-COBS rats (Charles River, Italy) were used, weighing 125–150 g at the beginning of the experiment. Animals were housed in standard conditions.

[D-Ala^2] methionine enkephalinamide (DALA), a synthetic analog of the naturally occurring opioids, and morphine hydrochloride were dissolved in saline and injected in the lateral cerebral ventricle at doses of 50 μg in 10 μl (DALA) and 25 μg in 10 μl (morphine) through two polyethylene cannulas implanted 3 days before. In a third experimental group, morphine hydrochloride was dissolved in saline and injected IP at a dose of 15 mg/kg. One hour after intracerebral DALA and morphine injections, and two hours after peripheral morphine treatment, rats were rapidly killed by decapitation using a guillotine, and the body was immediately placed in a 5 l. glass beaker. The time between first and last clonic movement was recorded with a stopwatch as the PDR.

Next, the spinal cord was quickly removed from C_2–C_3 to T_5 to T_6 (13–14 segments, mean tissue weight 250 mg), frozen on dry ice and kept at −80°C for biochemical determination. Dopamine and serotonin metabolites homovanillic acid (HVA), 3,4 dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA), were determined using

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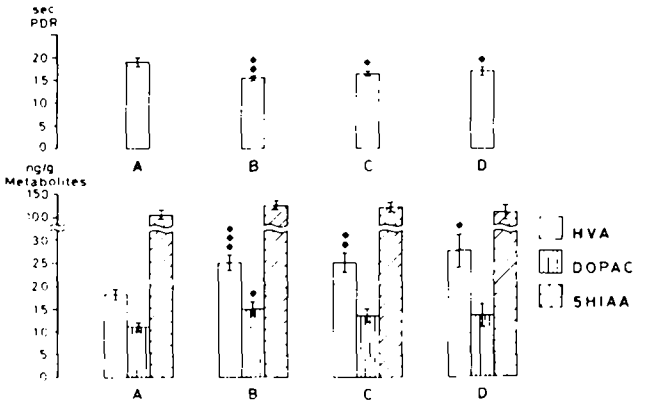

FIG. 1. The effect of opiate drug treatment on duration of the postdecapitation reflex (PDR) (upper part of the figure) and on HVA, DOPAC and 5HIAA (lower part of the figure) in the rat spinal cord. The data are mean + S.E. A—control, saline injected rats, n = 12; B—IP morphine injected rats, n = 14; C—ICV DALA injected rats, n = 12; D—ICV morphine injected rats, n = 6; $*p<0.05$; $**p<0.02$, $***p<0.01$ by Duncan's new mutliple range test.

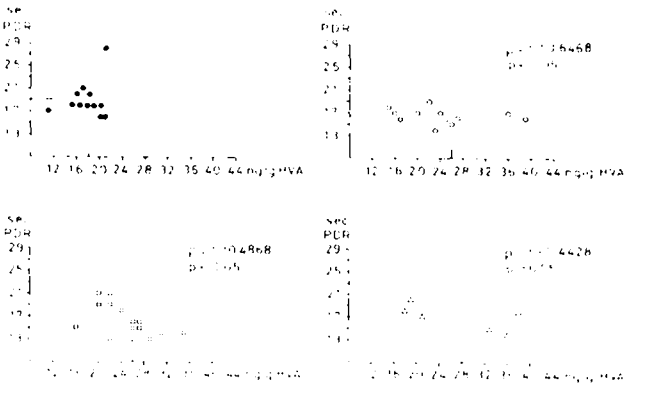

FIG. 2. Correlation between the effects of opiate treatment on PDR and the levels of HVA in S.C. Ordinate: duration of PDR in sec. The average value is indicated by the segment perpendicular to the vertical axis. Abscissa: HVA concentration ($\mu g/g$). The average value is indicated by the segment perpendicular to the horizontal axis. Solid circles = rats treated with saline; open circles = rats treated with morphine (IP); squares = rats treated with DALA (ICV); triangles = rats treated with morphine (ICV). The significance of correlation was analyzed using a Spearman's rank correlation coefficient.

liquid chromatography with electrochemical detection (LCED) as described by Ponzio and Jonsson [18]. The values were expressed in nanograms per gram of tissue.

The experiment was run in two parts; in each part treated groups were balanced by appropriate saline injected control groups. All behavioural and biochemical data were taken from the same rats; there were at least 6 animals in each experimental group.

Significance of behavioral and biochemical data was calculated using Duncan's new multiple range test. To assess the correlation between the biochemical and behavioral effects of opiate treatment the Spearman rank correlation coefficient was used [15].

RESULTS

Acute morphine and acute DALA treatment significantly shortened the duration of the PDR and the effect was even more pronounced when morphine was given systemically (Fig. 1). In the same rats 120 min after peripheral morphine injection there was a significant rise in the levels of the metabolites HVA and DOPAC in the S.C. DALA and central morphine treatment, after 60 min, induced a significant rise only in the concentration of HVA, compared with the control group (Fig. 1). None of the treatments changed the concentration of the 5HT metabolite 5HIAA, although some tendency to accumulation was present (Fig. 1). The Spearman rank correlation coefficient showed a significant negative correlation between biochemical and behavioral effects of peripheral morphine treatment ($\rho = 1\text{–}10{,}4868$, $p<0.05$) and centrally injected DALA ($\rho = 1\text{–}10{,}6468$, $p<0.05$).

In the centrally injected morphine group, the correlation coefficient showed the same tendency as in other treated groups, but did not reach the level of significance ($\rho = 1\text{–}10{,}4428$, $p>0.05$) probably because of the limited number of samples in this group (Fig. 2).

DISCUSSION

It has been reported that DA has a role of its own in the S.C. and is not merely a precursor of NE [5,11]. Our results indicate that in the S.C. DA undergoes oxidative metabolism similar to that in the CNS, with the exception that here the ratio of HVA to DOPAC is higher than in the CNS. The reason for this is not clear but it might arise from a difference in metabolite elimination processes or in the activities of MAO and COMT enzymes or through a contribution of telencephalic HVA transported via cerebrospinal fluid into the S.C.

Morphine and opiate peptides are known to cause dose-dependent stimulation of the metabolism of DA and 5HT, and this is reflected by an increase in the concentrations of their metabolites in many structures of the forebrain [1, 2, 3, 4, 7, 9, 12, 20].

This effect has been shown to be due to opiate drugs increasing both synthesis and the intraneuronal exposure of the neurotransmitter to the metabolizing action of monoamine oxidase as a consequence of its reduced release [1, 2, 7, 12]. In our experiment, morphine and DALA significantly raised the levels of DA metabolites in the S.C. too. The effect of IP injected morphine seemed to be stronger than that of DALA and concerned both DA metabolites. It should be borne in mind however, that parenterally morphine treated rats were decapitated 60 min later than DALA and centrally morphine injected rats, which could help explain the greater accumulation of DA metabolites in this group. Another possibility is that morphine, once injected peripherally, reaches the receptor sites in the S.C. more easily than opioids injected into cerebral ventricles. Opiate treatment did not significantly raise the concentration of 5HIAA in the S.C. in spite of a tendency to do so. Published data so far indicate that in fact 5HT neurons react less strongly than DA neurons to opiates [3,8]. The findings presented here substantiate the general conclusion of similar features of telencephalic and spinal DA and 5HT systems, as regards their reaction to some groups of psychotropic drugs.

Treatment with opiate drugs influenced the PDR. After acute morphine and DALA treatment the PDR was significantly shortened, to a similar degree (about 20%) in all exper-

imental groups. The Spearman rank correlation coefficient showed that the inhibitory effect of opiates on PDR was strongly correlated with its effect on DA neurons. Although significant, this effect was not as strong as that found by others after lesioning catecholaminergic neurons with 6-hydroxydopamine (6-OHDA) [17]. Possibly, the effect of the lesion on PDR is stronger than that elicited by opiate treatment. Moreover, 6-OHDA lesions also affect NA while at this time we do not know how opiates affect this monoamine in the S.C.

It has been demonstrated that selective depletion of brain DA may significantly inhibit the PDR [15]. It has also been shown that narcotic drugs are ineffective on the S.C. NE system [6]. These and our results suggest therefore that the DA system in the S.C. plays some role in PDR.

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