Psychopharmacology (1998) 139 : 251–254

ORIGINAL INVESTIGATION

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Repeated heroin administration increases extracellular opioid peptide-like immunoreactivity in the globus pallidus/ventral pallidum of freely moving rats

Received: 6 October 1997 / Final version: 2 March 1998

Abstract Microdialysis was used to investigate the effects of heroin administration on extracellular opioid peptide levels in the globus pallidus/ventral pallidum of freely moving rats. Two injections of heroin (0.6 mg/kg IP) were given 3 h apart. The first injection had no significant effect on opioid peptide levels, but the second injection produced a transient yet significant increase (+268%) in opioid peptide-like immunoreactivity in pallidal dialysates, peaking 1 h after injection. This effect was blocked by administration of naloxone (3 mg/kg IP) prior to the second injection. The implications of these data are discussed with regards to the role of the endogenous opioid peptide system in opiate reward.

Key words Enkephalin · Globus pallidus · Ventral pallidum · Opiates · Microdialysis · Heroin

Introduction

The pallidum, comprised of the globus pallidus and ventral pallidum, receives dense enkephalinergic innervation from the dorsal and ventral striatum (Cuello and Paxinos 1978; Zahm et al. 1985), areas which themselves receive equally dense dopaminergic innervation from the midbrain. Activation of mesolimbic dopamine neurons in particular is thought to be responsible for the rewarding actions of opiate drugs (see Di Chiara and North 1992; Koob 1992 for reviews). However, the enkephalins have also long been suspected of mediating opiate reward (Hughes 1976; Kosterlitz and Hughes 1976), and the effectiveness of lesions of the pallidum in blocking opiate self-administration (Hubner and Koob 1990) lends credence to this hypothesis.

Microdialysis studies from our laboratory have previously shown that systemically administered morphine induces a dose-dependent and site-specific increase in pallidal extracellular opioid peptides, primarily Met- and Leu-enkephalin, in freely moving rats (Olive et al. 1995). The present study sought to determine if heroin, a widely abused opiate drug and metabolic precursor to morphine, could similarly induce an increase in pallidal extracellular opioid peptides.

Materials and methods

All experimental procedures were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85–23, revised 1985). Adult male Sprague-Dawley rats (250–350 g) were anesthetized with halothane in a 1:1 mixture of O_2 and N_2O and implanted with guide cannulae to a depth of 2 mm above the globus pallidus/ventral pallidum (stereotaxic coordinates AP: −0.8 mm, ML: ± 2.9 mm, DV: −3.0 mm from bregma according to atlas of Paxinos and Watson (1986)) and secured with skull screws and dental cement. Animals were allowed to recover for 3–6 days prior to dialysis probe implantation. Following recovery, animals were lightly reanesthetized as described above and implanted with CMA/12 microdialysis probes with 4 mm polycarbonate membranes (10000 molecular weight cut-off) to a final depth of −9.2 mm from the skull surface. Probes were continuously perfused with an artificial cerebrospinal fluid (aCSF), containing 125 mM NaCl, 2.5 mM KCl, 0.5 mM NaH_2PO_4 (H_2O), 5 mM Na_2HPO_4, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$, 5 mM D-glucose, 0.2 mM L-ascorbic acid and 0.025% (w/v) bovine serum albumin, pH 7.3–7.5) at a rate of 2.0 µl/min. Probes were secured with dental cement and attached to dual channel liquid swivels for freely moving microdialysis procedures. Animals were allowed to recover from probe implantation for at least 12 h prior to pharmacological experiments. On the following day, drugs were dissolved in 0.1 M phosphate-buffered saline (pH = 7.4) and injected IP. Animals were kept under a 12:12 light/dark cycle (lights on 0700 hours) with free

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access to food and water. All experiments were performed during the lights-on phase.

Dialysis samples were collected at a flow rate of 2.0 µl/min every 30 min at room temperature and then stored at −70°C prior to radioimmunoassay procedures. At the end of each experiment, animals were deeply anesthetized with Nembutal (150 mg/kg IP). Brains were then removed, cut into 30 µm coronal sections, and stained with cresyl violet for verification of dialysis probe placement. Data from animals with probes found to be outside of the globus pallidus/ventral pallidum were discarded from the study.

A highly sensitive solid-phase "universal" opioid peptide radioimmunoassay was used to analyze pallidal dialysate opioid peptide content, as decribed elsewhere (Maidment et al. 1989; Maidment and Evans 1991). The detection limit of this assay was 0.1 fmol, and the ED_{50} was approximately 1.0 fmol. Although this assay recognizes all three major classes of endogenous opioid peptides, previous HPLC analysis has shown the primary peptides recovered from pallidal dialysates to be Met- and Leu-enkephalin (Maidment et al. 1989; Maidment and Evans 1991).

Drug-induced changes in dialysate opioid peptide-like immunoreactivity are presented as a percentage of pre-injection control levels computed as the average amount of immunoreactivity recovered per 30 min sample over a 3-h period prior to the first pharmacological intervention. Statistical analysis was conducted on the raw (fmol/30 min) data using a two-way repeated measures analysis of variance (ANOVA) followed by one-tailed Dunnett's post hoc tests with the sample immediately prior to each heroin administration as the control comparison value. *P*-values <0.05 were considered significant.

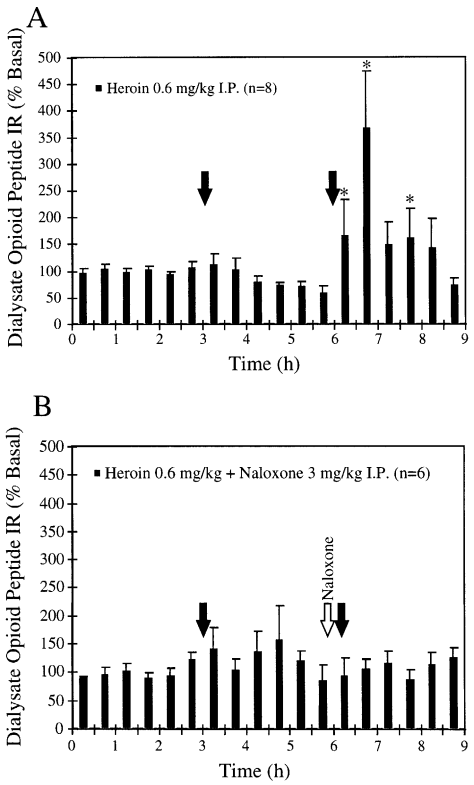

Fig. 1 A The effect of two sequential injections of heroin (0.6 mg/kg IP) on pallidal dialysate opioid peptide immunoreactivity ($n = 8$). **B** Blockade of the heroin-induced increase by naloxone (3 mg/kg IP) injected 10 min prior to the second heroin injection ($n = 6$). *$P < 0.05$ versus sample taken immediately prior to the second heroin injection

Results

The basal dialysate level of opioid peptide-like immunoreactivity averaged over the initial 3 h pre-injection period was 0.42 ± 0.04 fmol/30 min (Mean ± SEM). There was no significant diference in basal levels between the group receiving heroin (0.6 mg/kg, IP) alone and the group receiving naloxone (3 mg/kg, IP) prior to the second heroin injection. Heroin injection induced an increase (+268 ± 107%, Mean ± SEM) in dialysate opioid peptide-like immunoreactivity after the second but not the first injection, an effect which peaked during the second 30-min sample after injection (Fig. 1A) and which was blocked by administration of naloxone immediately prior to the second injection (Fig. 1B). Thus, statistical analysis (ANOVA) revealed a significant effect of time across the two groups ($F_{1,17} = 2.7$, $P < 0.05$) and a significant time versus group interaction ($F_{2,17} = 3.2$, $P < 0.05$). Post-hoc analysis showed no significant effect of the first heroin injection in either group, but revealed a significant increase after the second heroin injection.

Discussion

Heroin and other abused drugs are known to produce a substantial increase in dopamine release from the terminals of the mesolimbic pathway (Di Chiara and Imperato 1988; Hemby et al. 1995; Wise et al. 1995), which is believed to be a common neurobiological substrate for various classes of abused substances (Di Chiara and Imperato 1988; Koob 1992). Indeed, some studies have indicated that heroin reward is dependent on an intact mesolimbic dopamine system (Bozarth and Wise 1981; Spyraki et al. 1983). However, there is a growing body of evidence demonstrating that the rewarding effects of heroin may be independent of the dopaminergic system (Pettit et al. 1984; Van Ree and Ramsey 1987; Dworkin et al. 1988; Gerber and Wise 1989), but rather are dependent on the endogenous opioid system (Ettenberg et al. 1982; Vaccarino et al. 1985). Microinjection studies suggest that the pallidum, which is the enkephalinergic output target of the striatum, may be involved in the rewarding effects of opiate drugs (Johnson et al. 1993; Johnson and Stellar 1994). Moreover, lesions of the ventral portion of the pallidum will attenuate heroin self-administration (Hubner and Koob 1990).

Previous studies from our laboratory have shown that morphine (10 mg/kg IP) induces a substantial increase in pallidal extracellular opioid peptide levels (Olive et al. 1995). We have now demonstrated that heroin induces a naloxone-reversible increase in pallidal extracellular opioid peptide content similar in magnitude and duration to that seen with morphine, but

only after two serial injections. Whether this elevation reflects an increase in pallidal opioid peptide release [presumably enkephalins on the basis of our previous data (Maidment et al. 1989)] or reflects changes in metabolism or clearance of such peptides cannot be determined from our current data. Moreover, the possibility that we are sampling changes in such measures originating outside of the pallidum, in the striatum for instance, cannot be ruled out. Since activation of opioid receptors in the pallidum produces a hyperlocomotor response (Joyce et al. 1981; Dewar et al. 1985; Austin and Kalivas 1990; Hoffman et al. 1991; Napier 1992), the possibility also exists that this behavioral activation itself is responsible for the increased pallidal opioid peptide levels. However, since the neurochemical response was only apparent after the second heroin injection and we failed to observe overt differences in the behavior of the animals between the first and second injection, this explanation appears unlikely.

The reason for the lack of effect of the first injection is currently unclear. Peripheral (IP) injection of morphine induced an increase in pallidal dialysate opioid peptides within 30 min. of administration (Olive et al. 1995). Heroin itself has been shown to have poor binding affinity for opioid receptors (Inturrisi et al. 1983), and is thought to exert its effects after biotransformation to 6-monoacetylmorphine (MAM), morphine or morphine-6-glucuronide (M6G) (Way et al. 1960; Umans and Inturrisi 1981; Inturrisi et al. 1983; Rady et al. 1991, 1994; Rossi et al. 1996), which are active at μ and/or δ opioid receptors (Rady et al. 1991). It is therefore possible that repeated injections of heroin are necessary to accumulate enough heroin metabolites (i.e., MAM, morphine, M6G) in the pallidum to produce the observed elevation in extracellular opioid peptide immunoreactivity. Indeed, recent data from our laboratory has shown that local pallidal administration of nanomolar concentrations of M6G or morphine elicits a substantial increase in extracellular opioid peptides in this structure (Olive and Maidment 1996). It is also not possible with our current experimental design to rule out the possibility that the observed increase is a delayed effect of the first injection alone, but the fact that delayed administration of naloxone blocked the increase tends to argue against this interpretation.

An alternative explanation for the delayed effect would invoke an acute sensitization process. Interestingly, whereas conditioned place preference has been demonstrated following a single trial with morphine, amphetamine, or cocaine (Mucha et al. 1982; Bardo and Neisewander 1986; Bardo et al. 1986; Parker 1992), studies reporting place preference for heroin after a single administration are difficult to find; three or four trials being the norm (Spyraki et al. 1983; Schenk et al. 1985; Amalric et al. 1987; Hand et al. 1989; Stinus et al. 1989; see Bardo et al. 1995 for review), Although such trials are generally spaced at 24- or 48-h intervals rather than the 3 h employed in the present study it is nevertheless tempting to speculate that induction of endogenous opioid peptide release may be correlated to the acquisition of heroin reinforcement. Clearly, further studies are warranted to test this hypothesis.

Acknowledgements This study was supported by NRSA Predoctoral Fellowship DA 05634 to MFO, PHS grants DA 05010 and DA 09359, and the Hatos Foundation.

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