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Effects of the intravenous administration of [Lys^7]dermorphin on local cerebral glucose utilization in the rat

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Abstract

The use of analgesic opioids in the clinical setting is hampered by the reinforcing and addictive properties of these drugs. Moreover, chronic administration of conventional opioids is accompanied by progressive reduction of the analgesic effects, that often forces clinicians to increase dosages, exposing a subject to serious side-effects. Thus, interest is growing in the development and characterization of synthetic opioid agonists with lower reinforcing properties than conventional opioids. [Lys^7]dermorphin is a μ1 receptor agonist with 20–30 times stronger analgesic properties than morphine. Previous data indicate that the drug causes fewer side-effects than conventional opioids, and is less likely to produce physical dependence than morphine. In this study we investigated the effects of the intravenous administration of a range of doses of [Lys^7]dermorphin (0.002, 0.01 and 0.05 mg/kg) on local cerebral glucose utilization in the rat, by means of the quantitative [^{14}C]2-deoxyglucose method. The results of the study showed dose-related reductions of cerebral metabolic rates for glucose in limbic, sensory-motor and autonomic regions following the intravenous administration of [Lys^7]dermorphin. Such pattern of changes is similar to those measured earlier following the administration of analgesic doses of drugs stimulating μ-opioid receptors. Within the nucleus accumbens, and the shell portion in particular, we did not measure any increase of glucose utilization, rather a significant decrease following the administration of the higher dose of [Lys^7]dermorphin. These findings contribute to the definition of the functional consequences of the administration of [Lys^7]dermorphin, and indirectly suggest the lack of effect of the drug on mesolimbic dopamine neurotransmission.

Keywords: [Lys^7]dermorphin; Deoxyglucose; Cerebral metabolism; Analgesia

1. Introduction

The analgesic properties of conventional opioids are currently exploited in the clinical setting, but the use is hampered by the reinforcing and addictive properties of these drugs. Furthermore, chronic administration of conventional opioids is accompanied by progressive reduction of the analgesic effects, that often forces clinicians to increase dosages, exposing the subject to serious side-effects (Eisenberg et al., 2005). Thus, interest is growing on the development and characterization of opioid agonists with lower reinforcing properties than conventional ones. Extensive *in vitro* and *in vivo* studies have demonstrated that dermorphins are the most potent and selective μ-opioid receptor agonists among the naturally occurring opioids (Melchiorri and Negri, 1996). In particular, [Lys^7]dermorphin shows an affinity and selectivity for μ-opioid receptors of the rat brain membrane 10 times higher than dermorphin and 100 times higher than morphine. It has a relatively high rate of blood-to-brain influx and displays a saturable brain efflux (Negri et al., 1995). The antinociceptive effects of the systemic administration of [Lys^7]dermorphin are 20–30 times stronger than those of morphine (Negri et al., 1995). In addition to antinociception, the intracerebroventricular injection of [Lys^7]dermorphin causes dose-related effects on locomotor activity in the rat; low doses of the peptide stimulate locomotor behavior,

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whereas higher doses cause catatonia (Paakkari et al., 1990). In rats and mice, central or peripheral administration of dermorphin-like peptides induces a significantly slower development of tolerance to the antinociceptive effects than does morphine (Broccardo et al., 1985; Negri et al., 1995). Moreover, withdrawal symptoms precipitated by naloxone injection are considerably less severe in rats exposed to dermorphins than in animals receiving morphine (Broccardo et al., 1985). Based on those previous data, [Lys^7] dermorphin may represent a useful antinociceptive drug.

Experimental data indicate that the reinforcing properties of opioids are at least in part mediated through activation of the mesolimbic dopamine system (Di Chiara et al., 2004; Przewlocki, 2004). To this respect, the intravenous (i.v.) administration of morphine or heroin, at single unit doses corresponding to those that sustain self-administration, consistently increases extracellular dopamine concentrations in the shell of the rat nucleus accumbens (Pontieri et al., 1995; Tanda et al., 1997). This neurochemical effect is accompanied by stimulation of energy metabolism in the nucleus accumbens (Orzi et al., 1996; Martin et al., 1997), as measured by the quantitative autoradiographic [^{14}C]2-deoxyglucose method (2-DG) (Sokoloff et al., 1977). Conversely, reductions of energy metabolism in the nucleus accumbens were measured following systemic administration of higher, analgesic, doses of opioids (Beck et al., 1989; Cohen et al., 1991; Della Puppa et al., 1993; Ableitner, 1994; Fabian and Ableitner, 1995; Suzuki et al., 1997). In the present study, we applied the 2-DG method to investigate the effects of the acute i.v. administration of a range of doses of [Lys^7]dermorphin on local rates of cerebral glucose utilization in freely moving rats. In particular, we meant to test whether the drug produces a pattern of metabolic changes at the level of the terminal areas of the mesolimbic dopamine pathway different from those associated with the administration of reinforcing doses of conventional opiates.

2. Materials and methods

The experiments were carried out on male Sprague–Dawley rats weighing 270–300 g (Charles River, Italy). The animals were housed in group cages under standard temperature and humidity, on a 12-h light/dark cycle (light on 07.00:19.00), with free access to food and water. All procedures were carried out according to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health, and approved by the Local Ethical Committee. [Lys^7]dermorphin was synthesized as previously described (Negri et al., 1992). Drug or vehicle was administered i.v. in a volume of 0.2 ml/kg of body weight.

On the morning of the experiments, the femoral vessels of each rat were catheterized under halothane anesthesia, according to Crane and Porrino (1989). The animals were allowed at least 3 h to recover from anesthesia, and were then divided into four groups (n=3 each), and treated with either [Lys^7]dermorphin (0.002, 0.01, or 0.05 mg/kg) or vehicle. The 2-DG method was begun 5 min after the administration of drug or vehicle, by injecting a pulse of [^{14}C]2-DG (100 μCi/kg, specific activity 50–55 mCI/mmol, Amersham, UK) through the venous catheter. The remainder of the procedure was carried out according to the original procedure (Sokoloff et al., 1977), with the adaptation for use in freely moving rats (Crane and Porrino, 1989). Approximately 45 min after the administration of the tracer, the animals were sacrificed by the i.v. injection of sodium pentobarbital, the brains were rapidly removed, frozen in isopentane at −40 °C, and stored at −80 °C until sectioning. Cryostatic coronal sections

Table 1
Effects of the intravenous administration of [Lys^7]dermorphin on local cerebral glucose utilization (μmol/100 g/min) in the rat

Structure	0.000 mg/kg	0.002 mg/kg	0.01 mg/kg	0.05 mg/kg
Medial prefrontal cortex	69±5	59±8	67±11	65±4
Lateral prefrontal cortex	86±7	73±5	70±8[a]	66±3[b]
Nucleus accumbens (shell)	77±7	70±3	67±1	63±4[a]
Nucleus accumbens (core)	81±5	79±5	77±4	69±2[a]
Olfactory tubercole	91±7	85±5	87±3	73±3[b]
Caudate–putamen (dorsolateral)	104±9	102±5	96±6	85±6[a]
Caudate–putamen (dorsomedial)	110±10	108±4	98±5	83±6[b]
Caudate–putamen (ventral)	93±9	93±6	84±6	75±5[a]
Sensory-motor cortex	90±10	85±4	85±6	78±5
Lateral septum	58±5	50±3	45±4[a]	41±4[b]
Medial septum	75±10	65±5	64±6	58±3[a]
Anterior cingulate cortex	104±10	99±10	86±8	77±8[a]
Bed nucleus striae terminalis (dorsal)	44±4	43±2	38±3	37±5
Bed nucleus striae terminalis (ventral)	41±4	42±3	37±4	36±5
Globus pallidus (ventral)	57±8	54±4	45±6	38±6[a]
Globus pallidus (dorsal)	57±8	59±5	51±1	46±3
Subthalamic nucleus	83±13	83±9	77±2	74±6
Thalamus (anteroventral)	133±19	121±8	94±7[a]	87±9[b]
Thalamus (posteroventral)	52±5	45±3	41±2[a]	38±3[b]
Thalamus (ventroposterolateral)	95±15	89±11	81±4	74±5
Thalamus (ventromedial)	106±12	101±9	93±8	85±5
Thalamus (ventrolateral)	94±5	85±7	83±8	78±3[a]
Thalamus (mediodorsal)	114±17	119±5	106±9	78±4[a]
Hypothalamus (dorsomedial)	55±5	43±5[a]	41±1[a]	35±4[b]
Hypothalamus (paraventricular)	48±4	40±3[a]	39±1[a]	33±4[b]
Hypothalamus (lateral)	55±8	49±5	46±3	39±3[a]
Amygdala (central)	45±6	44±4	42±4	33±4[a]
Amygdala (basolateral)	74±9	78±9	70±6	63±5
Habenula (medial)	70±9	66±6	63±3	62±6
Habenula (lateral)	107±23	108±7	94±6	86±10
Hippocampus (CA1)	61±8	60±4	59±1	54±2
Hippocampus (CA2)	66±10	65±4	60±1	56±4
Hippocampus (CA3)	73±11	67±4	61±1	55±5[a]
Hippocampus (CA4)	59±7	54±5	52±1	51±4
Dentate gyrus	67±8	69±3	62±2	59±4
Substantia nigra compacta	63±5	64±5	63±3	56±4
Substantia nigra reticulata	59±4	59±2	58±1	49±2[b]
Ventral tegmental area	63±7	65±3	61±1	51±3[a]
Auditory cortex	131±20	131±11	127±12	131±23
Medial geniculate body	116±20	115±12	107±10	116±9
Inferior colliculus	129±12	134±14	132±10	124±8
Visual cortex	78±7	74±4	73±6	68±6
Lateral geniculate body	72±9	72±5	75±5	68±3
Superior colliculus (external layer)	72±10	77±3	72±3	64±5
Superior colliculus (deep layer)	80±11	82±3	75±8	67±5
Pontine gray	48±4	42±3	37±2[a]	36±4[b]
Medial raphe nucleus	72±6	65±4	64±5	60±6
Cerebellar cortex	47±4	44±5	43±3	38±3[a]

Data are expressed as means±SD, n=3 for each group. [a]$P<0.05$, [b]$P<0.01$ different from control (0.000 mg/kg), Dunnett's t-test statistic.