ELSEVIER

European Journal of Pharmacology 335 (1997) 133–137

ejp

Functional correlates of heroin sensitization in the rat brain

Francesco E. Pontieri [a,*], Laura Calò [a], Renato Di Grezia [b], Francesco Orzi [b], Francesca Passarelli [a]

[a] *Department of Neuroscience, University 'La Sapienza', Viale dell'Università 30, 00185 Rome, Italy*
[b] *INM 'Neuromed', Pozzilli (IS), Italy*

Received 10 June 1997; revised 18 July 1997; accepted 22 July 1997

Abstract

The aim of the study was to measure the changes in cerebral energy metabolism and c-*fos* mRNA expression following challenge with heroin in drug-naive rats and in animals previously sensitized to the drug. Acute heroin administration to drug naive-rats produced a generalized metabolic depression. In contrast, challenge with heroin in drug-sensitized rats produced selective metabolic increases in structures belonging to the basal ganglia. These changes were accompanied by increased c-*fos* mRNA expression in the caudate-putamen nucleus. These results demonstrate that the process of sensitization to heroin is coupled to functional changes that are confined to the subcortical motor circuits of the basal ganglia. © 1997 Elsevier Science B.V.

Keywords: Heroin; Sensitization; Deoxyglucose; Basal ganglia; c-*fos*

1. Introduction

Chronic administration of opioids produces tolerance to the sedative effects of the drugs and enhancement of their stimulatory properties (Nestler, 1996). This latter phenomenon, termed behavioral sensitization, has been observed with different classes of drugs of abuse (Kalivas et al., 1988) as well as with drugs that do not have addictive properties, such as apomorphine (Rowlett et al., 1991).

In the case of opioids, it has been suggested that the acute motor stimulant effects are mediated by the activation of opiate receptors located within the mesolimbic dopaminergic system, and a subsequent increase in dopamine release therein (Nestler, 1996). Evidence has also been provided that this same system is involved in the phenomenon of opioid-induced behavioral sensitization (Nestler, 1996). However, the increased motor activity characteristic of sensitization to opioids is accompanied by the occurrence of intense stereotypic behavior, and it has been suggested that functional activation of the nigrostriatal dopamine system is necessary to elicit stereotyped activity in experimental animals (for review, Orzi et al., 1993).

This study was performed to evaluate the cerebral functional changes underlying the process of behavioral sensitization to heroin in the rat. To address this issue, we used the [^{14}C]2-deoxyglucose method (Sokoloff et al., 1977) to measure the effects of the challenge with heroin on local rates of cerebral glucose utilization in rats previously sensitized to the drug. These effects were compared to those produced by the acute administration of heroin to drug-naive animals and to controls. Moreover, c-*fos* mRNA was detected in the caudate-putamen nucleus and sensory–motor cortex by reverse-transcription polymerase chain reaction (RT-PCR) in order to measure the effects of the treatment on the expression of an immediate early gene that is thought to play a role in the intracellular consequences of synaptic activation (Dragunow and Robertson, 1987).

2. Materials and methods

2.1. Local cerebral glucose utilization

The experiments were performed on male Sprague–Dawley rats (Charles River, Italy) weighing 250–275 g at the beginning of the treatment. The animals were housed in single cages under standard temperature and humidity,

* Corresponding author. Tel.: (39-6) 4991-4708; Fax: (39-6) 444-0790.

on a 12 h light/dark cycle (light on 07.00 a.m.–07.00 p.m.). They had free access to food and water.

Heroin (Salars, Italy) was dissolved in fresh saline +0.05% acetic acid. Heroin sensitization was accomplished by means of a three-day schedule of administration with progressively increasing doses of the drug. The rats were administered two subcutaneous (s.c.) injections per day, at 9.00 a.m. and 9.00 p.m.. The dose of heroin in each single injection was 5, 10, and 20 mg/kg, on day 1, 2, and 3, respectively. The control animals received an equivalent treatment with vehicle.

Two weeks after the end of the treatment with heroin or vehicle, the animals were lightly anesthetized with halothane (2% in oxygen) and polyethylene catheters were inserted into the femoral vessels, then tunnelled s.c. to exit at the nape of the neck. The rats were allowed at least 3 h to recover from anesthesia, before being treated with either heroin (1 mg/kg, s.c.) or vehicle. Acute heroin challenge was given to both drug-sensitized and drug-naive rats.

The [^{14}C]2-deoxyglucose experimental procedure began 5 min after the administration of heroin or vehicle, by means of the intravenous (i.v.) injection of a pulse of [^{14}C]2-deoxyglucose (100 μCi/kg, specific activity 50–55 mCi/mmol, Amersham, UK). Timed arterial blood samples were drawn according to the original method (Sokoloff et al., 1977), immediately centrifuged and plasma glucose and [^{14}C]2-deoxyglucose concentrations were measured. Approximately 45 min after the administration of the tracer, the rats were killed by the i.v. administration of a lethal dose of sodium pentobarbital, and the brains were removed, frozen in isopentane at −40°C and stored at −80°C until sectioning. Cryostatic coronal brain sections (20 μm) were dried on a hot plate and exposed to Kodak Min-R X-ray films (Kodak, Italy), along with a set of [^{14}C]methylmetacrylate standards (Amersham, UK). The resulting autoradiograms were analyzed using a computerized image-processing system (MCID, Imaging Research, Canada). Local tissue ^{14}C concentrations were measured from the optical densities and a calibration curve obtained from densitometric analysis of the autoradiograms of the calibrated standards. Rates of glucose utilization were then calculated according to the operational equation for the method (Sokoloff et al., 1977).

Rates of energy metabolism were measured in 41 discrete brain areas. The statistical analysis was carried out by using a one-way analysis of variance followed by Tukey's *t*-test for multiple comparisons.

2.2. c-fos mRNA

The RT-PCR method was used to estimate the relative changes of specific mRNA levels in the sensory–motor cortex and caudate-putamen nucleus of the animals. The rats were submitted to the same schedule of administration of heroin or vehicle as above ($n = 3$ for each group), and they were killed by decapitation 2 h after the challenge with drug or vehicle.

Total RNA was purified by the acid guanidinium thiocyanate–phenol–chloroform method (Chomczynski and Sacchi, 1987) followed by the elimination of genomic DNA with 2.5 u of RNase-free, pure DNase (Pharmacia, Italy). The reverse transcriptase reaction mixture (25 μl) consisted of 1 μg of total RNA per sample, and 200 U of Monoley Murine Leukemia Virus reverse transcriptase (M-MLV-RT, Pharmacia, Italy). Control amplification experiments were routinely performed on total RNA (1 μg) without carrying out the RT reaction. One-fourth of each cDNA sample was used in a PCR reaction mixture containing forward (F) and reverse (R) primers for the gene of interest and 'housekeeping' gene β-actin (Nudel et al., 1983). The location of c-*fos* primers was between positions (5′–3′) 338–838 (Curran et al., 1987).

The PCR amplification was performed in a final volume of 50 μl containing 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.05% Tween 20, 0.05% NP40, 1% dimethyl sulfoxide, 250 nM of each primer, 100 mM synthetic hexadeoxynucleotide primers, and 1–2 u of *Taq* DNA Polymerase (Promega, Italy). The amplification reactions were carried out with the following settings: denaturation at 94°C for 60 s, annealing at 60°C for 30 s, extension at 73°C for 30 s. The optimal number of cycles to allow detection in the linear phase of amplification was determined to vary between 22 and 26, using a Perkin Elmer-Cetus DNA thermal cycler (Italy).

The resulting PCR products were analyzed and the site of the amplified cDNA fragment corresponded to that predicted from the cDNA sequence and the set of primers used.

Semi-quantitative densitometric analysis of the PCR products was performed by using a computerized image processing system (MCID, Imaging Research, Canada). The system was calibrated for a set of optical density standards (Kodak, USA), and background subtraction was automatically performed. Data were analyzed as means ± S.D. Student's unpaired *t*-test was performed to evaluate the differences in the mean value of c-*fos*/β-actin ratio in the caudate-putamen and sensory–motor cortex of heroin-sensitized rats and control rats.

3. Results

3.1. Behavior

Catalepsy was observed following the administration of heroin to drug-naive rats. In contrast, challenge with heroin of drug-sensitized animals produced intense stereotypies, particularly licking, gnawing and biting activities. Increased locomotor activity was also observed, although precise measurement of locomotion was not accomplished.

3.2. Local cerebral glucose utilization

Local rates of cerebral glucose utilization in the different groups of rats are shown in Table 1.

3.2.1. Effects of heroin in drug-naive rats

The administration of heroin (1 mg/kg, s.c.) to drug-naive rats produced a generalized depression of brain energy metabolism. Significant decreases with respect to the control values were measured in limbic (medial prefrontal, anterior cingulate) as well as sensory–motor cortical areas. Energy metabolism was also reduced in the caudate putamen nucleus, in several thalamic nuclei, in the habenula (both medial and lateral portions), in the hippocampal complex, in the lateral and medial septal nuclei, and in the structures belonging to the visual and auditory pathways.

Table 1
Local cerebral glucose utilization (μmol/100 g per min) in rats treated with heroin

Structure	Control	Drug naive	Sensitized
Medial prefrontal cortex	73±2	58±2 [a]	73±4 [b]
Nucleus accumbens (shell)	80±3	61±4	77±8
Nucleus accumbens (core)	88±3	70±5	87±8
Anterior cingulate cortex	109±2	90±4 [a]	107±7 [b]
Sensory motor cortex	92±2	74±3 [a]	103±7 [b]
Caudate putamen (dorsolateral)	102±2	80±3 [a]	107±8 [b]
Caudate putamen (dorsomedial)	105±2	83±4 [a]	103±8
Caudate putamen (ventral)	89±2	80±4	99±8
Lateral septum	66±1	52±4 [a]	60±4
Medial septum	94±2	64±2 [a]	75±5 [a]
Globus pallidus (ventral)	80±1	60±4 [a]	70±2
Globus pallidus (dorsal)	54±2	48±4	59±7
Amygdala (basolateral nucleus)	86±3	64±3 [a]	91±5 [b]
Amygdala (central nucleus)	49±1	41±4	50±5
Thalamus (ventromedial nucleus)	110±3	79±3 [a]	114±9 [b]
Thalamus (ventrolateral nucleus)	85±3	64±3	101±9 [b]
Thalamus (anteroventral nucleus)	122±3	85±7 [a]	131±10 [b]
Entopeduncular nucleus	48±1	46±3	69±1 [a,b]
Thalamus (dorsomedial nucleus)	124±2	76±5 [a]	116±5 [b]
Thalamus (laterodorsal nucleus)	112±4	75±3 [a]	111±8 [b]
Habenula (medial)	76±3	55±2 [a]	70±3 [b]
Habenula (mediolateral)	98±3	67±3 [a]	86±3 [a,b]
Habenula (lateral)	109±3	76±3 [a]	99±3 [b]
Subthalamic nucleus	88±5	73±2	112±4 [a,b]
Lateral hypothalamus	61±3	48±4	59±1
Hippocampus (CA1)	69±3	48±5 [a]	72±4 [b]
Hippocampus (CA2)	85±5	56±4 [a]	85±3 [b]
Hippocampus (CA3)	77±6	53±5 [a]	77±2 [b]
Hippocampus (CA4)	66±4	46±5 [a]	66±3 [b]
Dentate gyrus	79±3	53±4 [a]	80±5 [b]
Lateral geniculate body	87±6	57±3 [a]	77±3 [b]
Auditory cortex	114±4	94±4 [a]	119±6 [b]
Medial geniculate body	108±4	83±4 [a]	106±6 [b]
Substantia nigra compacta	78±5	54±4 [a]	86±7 [b]
Substantia nigra reticulata	60±4	45±3	77±5 [a,b]
Ventral tegmental area	75±4	50±4 [a]	77±7 [b]
Visual cortex	98±5	72±4 [a]	96±4 [b]
Superior colliculus (external)	86±6	65±3	84±5
Superior colliculus (deep)	95±5	74±6	103±6 [b]
Inferior colliculus	122±6	100±10	127±7
Cerebellar cortex	61±4	40±5 [a]	79±6 [b]

Values represent means±S.E.M. ($n=4$ for each group).
[a] $p<0.05$ different from control; [b] $p<0.05$ different from drug-naive (Tukey's t test statistic).

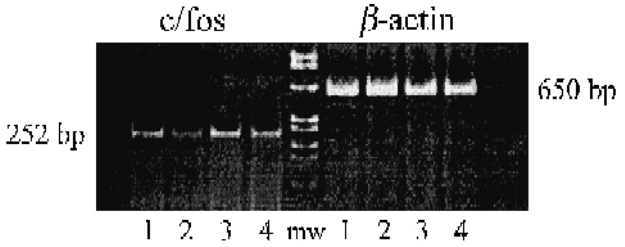

Fig. 1. RT-PCR reactions on the total RNA from the rat striatum and motor cortex, performed using oligonucleotide primers specific to the rat c-*fos* (left) and β-actin (right) transcripts. The specific oligonucleotides were used to generate 252 and 750 bp products, as indicated. Lane 'mw', 200 ng Pharmacia φX-174-RF DNA *Hae*III Digest. Lane 1 = caudate-putamen, heroin sensitized. Lane 2 = caudate-putamen, control. Lane 3 = sensory–motor cortex, heroin sensitized. Lane 4 = sensory–motor cortex, control. Reaction products were separated by electrophoresis on a 8% acrylamide gel and visualized with ethidium bromide staining. The lanes contained 8 μl of each amplification reaction product.

3.2.2. Effects of heroin in drug-sensitized rats

The effects of the same dose of heroin administered to drug-sensitized rats produced a markedly different pattern of changes in cerebral glucose utilization. In fact, significant increases with respect to control values were measured only in a few structures within the basal ganglia (subthalamic nucleus, entopeduncular nucleus, and substantia nigra pars reticulata). Rates of glucose utilization were lower than those measured in the controls in the medial septal nuclei. In all the remaining structures, the administration of heroin to drug-sensitized rats failed to modify energy metabolism with respect to control values. However, in most structures examined, the effects of the challenge with heroin in the drug-sensitized rats were significantly different from those measured in drug-naive rats.

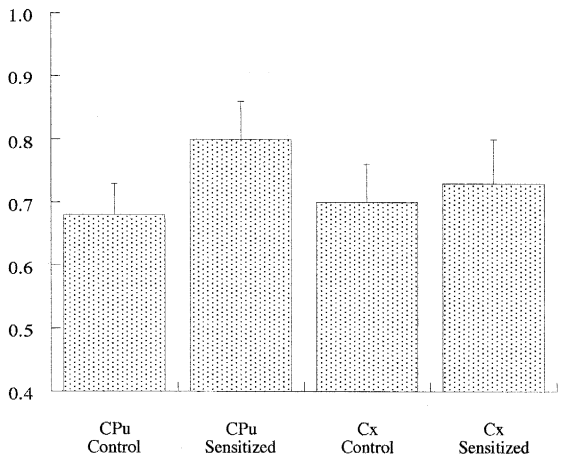

Fig. 2. c-*fos*/β-actin ratios in the caudate-putamen (CPu) and sensory–motor cortex (Cx) in heroin-sensitized rats and controls. Shown are means±S.D. for 3 animals in each group.

3.3. c-fos mRNA

Challenge with heroin of drug-sensitized animals produced a significant increase in the c-*fos* mRNA signal in the caudate-putamen (+21% with respect to control, $P < 0.05$) (Figs. 1 and 2). In contrast, a slight, not significant increase in the signal was measured in the motor cortex (+5% with respect to control).

4. Discussion

The results of the present study demonstrate that the s.c. administration of heroin produces different patterns of cerebral metabolic changes according to whether the recipient rats are drug-naive or drug-sensitized. In fact, a generalized depression of energy metabolism was measured following heroin challenge in drug-naive rats, whereas glucose metabolism was increased in selected brain areas of drug-sensitized animals.

The effects of a cataleptic dose of heroin in drug-naive rats were similar to those previously reported for morphine (London et al., 1986; Beck et al., 1989). These results, then, confirm that opioid-induced catalepsy in the rat is associated with reduced rates of cerebral glucose utilization. We previously reported that the i.v. administration of morphine, at doses corresponding to those that maintain self-administration in the rat, is associated with increased rates of glucose utilization in the shell of the nucleus accumbens (Orzi et al., 1996). The present report that acute high-dose heroin challenge in the rat fails to increase energy metabolism in the nucleus accumbens, and the shell in particular, is further evidence that metabolic activation in this area is the consequence of the administration of rewarding doses of opioids.

The administration of the same dose of heroin to rats previously sensitized to the drug produced a marked behavioral activation that was accompanied by a completely different pattern of changes in glucose utilization. Thus, the widespread reductions of energy metabolism measured following heroin administration to drug-naive rats were not seen. This phenomenon may reflect the functional equivalence of behavioral tolerance to the sedative effects of the drug. Metabolic activation was measured in selected brain areas of drug-sensitized rats. It is notable that the effects of heroin challenge in drug-sensitized animals were significant with respect to control values only in a few areas that are part of the motor circuitry of the basal ganglia (subthalamic nucleus, entopeduncular nucleus and substantia nigra pars reticulata). These results suggest that the behavioral sensitization to heroin, at least using the schedule of administration of the present study, is accompanied by functional changes that are mainly confined to the subcortical motor structures of the basal ganglia.

The [^{14}C]2-deoxyglucose method allows measurement of a dynamic biochemical process, energy metabolism, throughout the entire brain. The method is, however, unable to differentiate between direct and indirect effects of a given stimulus. An entire pathway or circuit may be metabolically activated even though the drug or stimulus may have a direct action only at the origin or at some point along the pathway. The results obtained following heroin challenge of drug-sensitized rats suggest that the effects of treatment are mainly localized within the motor circuitry of the basal ganglia. In order to better localize whether these changes are primarily at a striatal or cortical level, we measured c-*fos* mRNA expression. There is, in fact, evidence that the expression of c-*fos* mRNA is regulated by a variety of receptor-mediated processes, and that the proto-oncogene plays a role in the long-term consequences of synaptic activation (Dragunow and Robertson, 1987). Based on these considerations, the present observation that c-*fos* mRNA expression was significantly increased in the caudate-putamen but not in the sensory–motor cortex following heroin challenge of sensitized rats is further, indirect, evidence that the functional changes underlying the process of behavioral sensitization to heroin are mainly confined to the subcortical basal ganglia circuitry and that the caudate-putamen nucleus is likely to be the primary site in the brain where this process occurs.

The pattern of changes in glucose utilization measured following the administration of heroin to the drug-sensitized rats appears similar to that previously reported by us and other groups for high doses of dopamine agonist drugs (for review, Orzi et al., 1993). This similarity of effects on cerebral energy metabolism indirectly suggests that the process of sensitization to heroin is accompanied by an increased response of striatal neurons to nigrostriatal dopamine transmission. Whether this phenomenon reflects increased extracellular dopamine concentrations in the striatum or the enhancement of post-synaptic mechanisms of signal transduction cannot be defined on the basis of the present data.

A further relevant finding of this study is, in our opinion, the lack of effect of heroin challenge on the metabolic activity of the nucleus accumbens (shell and core subportions) in heroin sensitized rats. Previous reports from our laboratory (Pontieri et al., 1994, 1996; Orzi et al., 1996) suggest that increased metabolic activity in the shell of the nucleus accumbens is a common consequence of the acute administration of rewarding doses of drugs of abuse in the rat. Repeated treatment with cocaine is also accompanied by functional changes in the nucleus accumbens (Pontieri et al., 1995). In this respect, the lack of effect of heroin sensitization on the metabolic activity of the nucleus accumbens shown in our study suggests, in our opinion, that the functional changes that accompany the process of sensitization to heroin, at least under the conditions used in the present study, are confined to the motor structures of the basal ganglia and do not involve functional changes at the level of the mesolimbic dopamine system. If this is the case, our results suggest that the

process of sensitization to heroin is relatively independent of the rewarding properties of the drug.

Acknowledgements

This work was supported by funds from MURST. We would like to thank Mr. M. La Riccia for the excellent technical assistance and Professor C. Fieschi for the continuous and enthusiastic support.

References

Beck, T., Wenzel, J., Kuschinski, K., Krieglstein, J., 1989. Morphine-induced alterations of local cerebral glucose utilization in the basal ganglia of rats. Brain Res. 497, 205–209.

Chomczynski, P., Sacchi, N., 1987. Single step method for RNA isolation by acid-guanidiniumthiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Curran, T., Gordon, M.B., Rubino, K.L., Sambucetti, L.C., 1987. Isolation and characterization of the c-fos (rat) cDNA and analysis of post-transcriptional modification in vitro. Oncogene 2, 79–84.

Dragunow, M., Robertson, H.A., 1987. Kindling stimulation induces c-fos protein(s) in granule cells of the rat dentate gyrus. Nature 329, 441–442.

Kalivas, P.W., Duffy, P., DuMars, L.A., Skinner, C., 1988. Behavioral and neurochemical effects of acute and daily cocaine administration in rats. J. Pharmacol. Exp. Ther. 245, 485–492.

London, E., Fanelli, R., Szikszay, M., Jasinski, D., 1986. Effects of opioid analgesics on local cerebral glucose utilization. NIDA Res. Monogr. 75, 379–386.

Nestler, E.J., 1996. Under siege: The brain on opiates. Neuron 16, 897–900.

Nudel, U., Zakut, R., Shani, M., Neuman, S., Levy, Z., Yaffe, D., 1983. The nucleotide sequence of the rat cytoplasmic beta-actin gene. Nucleic Acids Res. 11, 1759–1771.

Orzi, F., Morelli, M., Fieschi, C., Pontieri, F.E., 1993. Metabolic mapping of the pharmacological and toxicological effects of dopaminergic drugs in experimental animals. Cerebrovasc. Brain Metab. Rev. 5, 95–121.

Orzi, F., Passarelli, F., La Riccia, M., Di Grezia, R., Pontieri, F.E., 1996. Intravenous morphine increases glucose utilization in the shell of the rat nucleus accumbens. Eur. J. Pharmacol. 302, 49–51.

Pontieri, F.E., Colangelo, V., La Riccia, M., Pozzilli, C., Passarelli, F., Orzi, F., 1994. Psychostimulant drugs increase glucose utilization in the shell of the rat nucleus accumbens. NeuroReport 5, 2561–2564.

Pontieri, F.E., Mainero, C., La Riccia, M., Passarelli, F., Orzi, F., 1995. Functional correlates of repeated administration of cocaine and apomorphine in the rat. Eur. J. Pharmacol. 284, 205–209.

Pontieri, F.E., Tanda, G., Orzi, F., Di Chiara, G., 1996. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382, 255–257.

Rowlett, J.K., Mattingly, B.A., Bardo, M.T., 1991. Neurochemical and behavioral effects of acute and chronic treatment with apomorphine in rats. Neuropharmacology 30, 191–197.

Sokoloff, L., Reivich, M., Kennedy, C., DesRosiers, M.H., Patlak, C.S., Pettigrew, K.D., Sakurada, O., Shinohara, M., 1977. The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure and normal values in the conscious and anesthetized albino rat. J. Neurochem. 28, 897–916.