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Systemic naloxone enhances cerebral blood flow in anesthetized morphine-dependent rats

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Abstract

Laser–Doppler flowmetry was used to study cerebral cortical blood flow responses to morphine and naloxone in morphine-naive and -dependent rats. The experiments were performed in spontaneously breathing anesthetized rats. Morphine (10 mg/kg, i.p.) administration reduced regional cerebral blood flow in control, sham-operated and morphine-dependent rats, but the depressant effect of morphine in morphine-dependent animals was less than that in control and sham-operated groups. While naloxone (0.5 mg/kg, s.c.) had no considerable effect on regional cerebral blood flow in control and sham-operated groups, it increased regional blood flow in morphine dependent ones. The depressant effect of morphine in all groups and the enhancing effect of naloxone in morphine-dependent animals were not seen after local application of lidocaine at the recording site. This study may provide a framework to study the cellular and molecular mechanisms responsible for coupling neuronal electrical activity with regional alterations in blood flow during precipitation of morphine withdrawal. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Morphine tolerance; Morphine dependence; Naloxone; Opiate withdrawal; Cerebral blood flow; Laser–Doppler flowmetry, (Rat)

1. Introduction

Continuing use of morphine is accompanied by pathological, psychological and physical dependence on the drug with the consequences of withdrawal after abrupt stopping of the drug (Wooten et al., 1982).Various and contradictory effects of natural and synthetic opioids on cerebral blood flow, cerebrovascular resistance, and cerebral metabolic rates of oxygen and glucose have been described. Results of these experiments suggest that synthetic opioids do not have a direct cerebrovascular action but that changes in cerebral blood flow are more related to metabolic, neuronal or respiratory effects of opiate receptor stimulation (Benyo and Wahl, 1996; Cohen et al., 1991; Turner et al., 1984). In spite of this, there are few studies exploring the changes in regional cerebral blood flow during opiate withdrawal (Krystal et al., 1995; Rose et al., 1996).

Clinical measurements of cerebral blood flow are currently performed using the Xenon-133 clearance technique or positron emission tomography (PET) (Arbit et al., 1989; Hauerberg and Juhler, 1997). Xenon-133 inhalation is technically complex to perform and there are limitations to its intraoperative use, including (1) long measurement times (10–30 min per measurement), (2) limited spatial resolution and (3) the necessity to account for recirculation of the tracer. PET, although a promising technique, is currently very costly and available in only a few centers. In addition, the limitations of PET technology are the same as those of the Xenon technique, namely, limited spatial resolution, the requirement of injection or inhalation of radioisotopes, and long measurement times (Arbit et al., 1989). One relatively recent technological advance for measuring blood flow is the development of Laser–Doppler flowmetry in which reflected laser light is used to determine changes in blood flow. This technique has a temporal resolution in the order of milliseconds, a variable spatial resolution (depending upon the width of the probe used), and collects flow data to a depth approximately 1 mm below the cortical surface (Fukuda et al., 1995; Gerrits et al., 1998). Therefore, the technique will provide new

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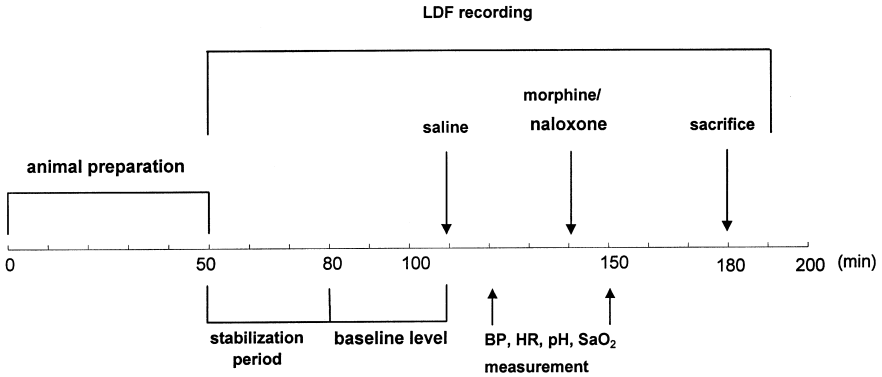

Fig. 1. Experimental protocol; timeline and order of experimental treatments and measures.

features that extend our knowledge of dynamic flow changes in the brain.

Previously, regional cerebral blood flow has been used as an index for neuronal activity (Gerrits et al., 1998; Hsieh et al., 1995). Lidocaine, as a local anesthetic agent, abolishes electrophysiological activity, and has been used for functional inactivation (Martin, 1991). Hence, using the application of lidocaine, we could investigate the effects of morphine and naloxone on neural activity.

The objective of the present study was to determine how regional cerebral blood flow is affected during naloxone administration which precipitated the morphine withdrawal syndrome in morphine-dependent rats.

2. Materials and methods

2.1. Animal preparation

Adult male Wistar rats (200–250 g) were used in all experiments. The animals were housed in groups of no more than six and maintained on pellets and water ad libitum. One group, as the sham-operated group, received tap water, the second group, as the control group, received 3% sucrose in tap water and the third group, as the dependent group, received morphine sulfate (Temad, Iran) and 3% sucrose in tap water. Rats were made dependent by chronic administration of morphine 0.1, 0.2 and 0.3 mg/ml each for 48 h and 0.4 mg/ml during the days for up to 21 days in their drinking water (Badavi and Evans, 1982; Haghparast et al., 1998). The withdrawal syndrome precipitated by naloxone was used as an indicator of the development of dependence on morphine.

Anesthesia was induced with urethane (Merck, 1.5 g/kg, i.p.) to allow tracheotomy. During the experiments, arterial blood pressure, arterial oxygen saturation (SaO_2), and pH were measured at various times. Arterial blood pressure was recorded invasively (Lafayette Instrument, USA) or non-invasively with an arterial catheter or programmed electro-sphygmomanometer (Narco Bio-System). Continuous arterial O_2 saturation values were obtained non-invasively with a pulse oximeter. Body temperature was kept within physiological ranges with a rat temperature control unit (Narco Bio-system).

2.2. Measurement of blood flow with Laser–Doppler flowmerty

The Laser–Doppler flowmeter (MBF3D, Moor instrument, Axminster, UK) used in this study was composed of

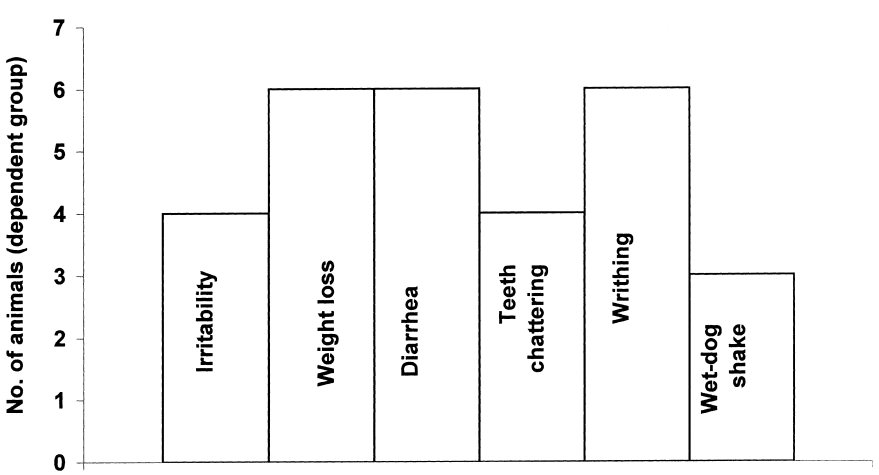

Fig. 2. Withdrawal signs precipitated by naloxone (1 mg/kg, i.p.) in morphine-dependent rats ($n = 6$).

Table 1
Physiological variables in rats of different groups for which cerebral blood flow was measured

Physiological parameters	Control			Sham			Dependent		
	S	M	N	S	M	N	S	M	N
MABP (mm Hg)	86.1 ± 0.33	85.6 ± 1.4	87.1 ± 3.5	85.36 ± 3	86.57 ± 2.6	84 ± 4.1	85.7 ± 1.9	84.1 ± 0.89	85 ± 2.01
H.R. (beats/min)	353 ± 19	345 ± 11.34	364.3 ± 9	337.5 ± 14.1	340 ± 10	338 ± 20	370.7 ± 12.5	363 ± 21	366 ± 12
pH	7.42 ± 0.02	7.41 ± 0.007	7.4 ± 0.01	7.4 ± 0.01	7.4 ± 0.01	7.4 ± 0.02	7.4 ± 0.02	7.38 ± 0.007	7.38 ± 0.02
A.O.S. (%)	95.5 ± 2.5	95 ± 3	5.66 ± 1.5	96.33 ± 2.2	6.4 ± 3.5	96.2 ± 1	95.36 ± 1.2	95.4 ± 2	95.2 ± 2.5

Values are reported as means ± S.E.M. ($n = 5$). Sampling time was 10 min post-injection of saline (S, 1 ml), morphine (M, 10 mg/kg) or naloxone (N, 0.5 mg/kg). MABP, mean arterial blood pressure; H.R., heart rate; A.O.S., arterial oxygen saturation.

a laser diode as a source (Wavelengh, 810 nm; output, 3 mW) and two optical fibers (one for emitting and the other for receiving laser light; external diameter = 0.46 mm). Separation of fibre centres was 0.25 mm. The animals were mounted in a stereotaxic frame and a 2-mm diameter hole was drilled in the skull above the parietal cortex (hindlimb area, 1.8–2 mm caudal to bregma, and 2.5 mm lateral to midline according to the atlas of Paxinos and Watson (1986). The dura matter was incised with a fine needle (30 gauge), with care being taken not to damage brain tissue. The probe was positioned with a micromanipulator perpendicular to the surface, avoiding large vessels. If bleeding occurred following probe placement, the rat was discarded from the study.

Recordings were allowed to stabilize for at least 30 min before obtaining baseline flow levels. Blood flow was monitored for 30 min following saline injection and subsequent measurements were converted to a percentage of the baseline control.

Morphine sulfate was given intraperitoneally (10 mg/kg, i.p.) to both morphine-naive and morphine-dependent rats after baseline recording. Naloxone hydrochloride (Sigma, USA) was given subcutaneously (0.5 mg/kg, s.c.) 30 min after saline injection (Fig. 1). Two additional groups of sham-operated and chronically morphine-treated rats received lidocaine (2%) (local application) simultaneously. During exposure of the pia mater, the brain surface was kept continually moist with artificial cerebrospinal fluid.

At the end of experiment, the animals were killed a with saturated solution of KCl injected intracardially and the biological zero values were measured (that LDF registered flow not equal to 0 for some time after cardiac arrest, this measuring was added). The biological zero values were subtracted from the flow values before calculation of percentage changes in blood flow.

2.3. Histological verification

Upon completion of each experiment, the recording site was marked by deposition of pontamine sky blue dye. The brains were removed and the recording sites were verified.

2.4. Data analysis

Student's paired *t*-test was used to determine the significance of the differences in CBF changes between various treatment groups. The data were subjected to two-way analysis of variance (ANOVA) followed by a protected Tukey's test for multiple comparisons, as needed. A $P < 0.05$ was considered to represent a significant difference.

3. Results

3.1. Test of dependence to morphine

Fig. 2 shows the results of the withdrawal syndrome test, precipitated by naloxone. Diarrhea, weight loss and writhing were common among all morphine-treated rats ($n = 6$). Control rats ($n = 4$) did not show any defined withdrawal signs.

3.2. Effects of morphine and naloxone on CBF

During the experiments, various physiological variables were either measured or checked (Table 1). Mean arterial blood pressure, SaO_2, pH and heart rate are reported from the data obtained 10 min after saline and 10 min after drug

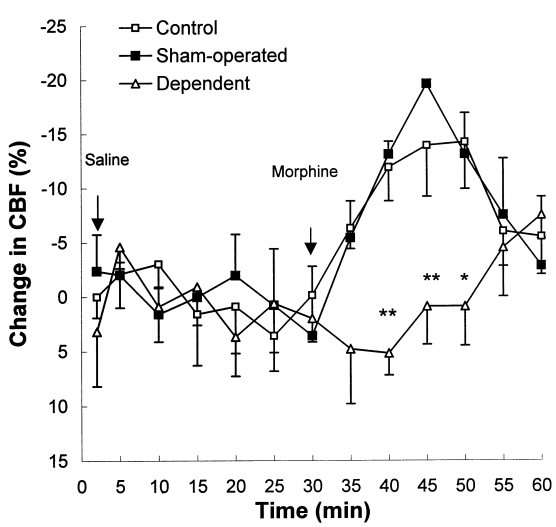

Fig. 3. Effects of morphine administration (10 mg/kg, i.p.) on regional cerebral blood flow in control, sham-operated and morphine-dependent, anesthetized rats. Values are expressed as means ± S.E.M. ($n = 6$). Data were subjected to two-way analysis of variance (ANOVA) followed by protected Tukey' s test for multiple comparisons. $^{*}P < 0.05$, $^{**}P < 0.01$, relative to control and sham operated animals.

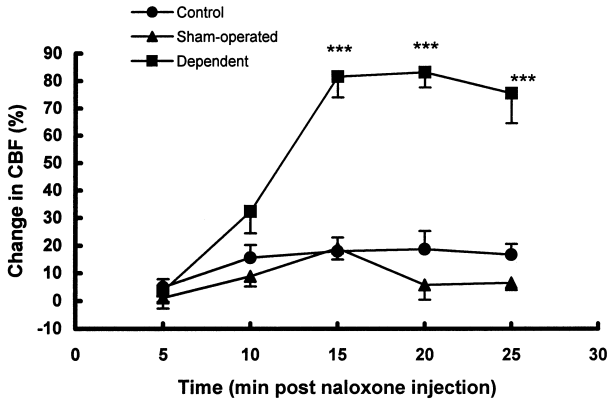

Fig. 4. The changes in cerebral blood flow during naloxone-induced withdrawal in morphine-dependent, anesthetized rats. The changes in CBF in control and sham-operated rats are also shown. Values are expressed as means ± S.E.M. ($n = 6$). Data were subjected to two-way ANOVA followed by protected Tukey's test for multiple comparisons. $^{***}P < 0.001$.

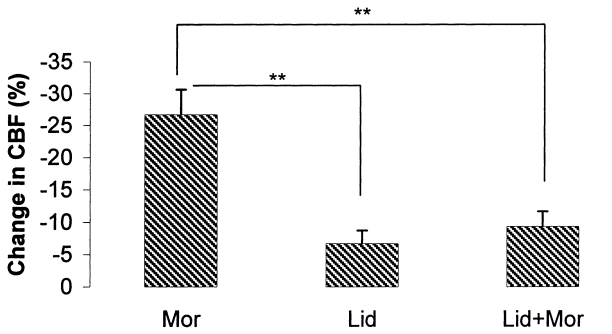

Fig. 6. Effects of local application of lidocaine (Lid) on rCBF after intraperitoneal administration of morphine (Mor, 20 mg/kg) in naive rats. Values are means ± S.E.M. from five rats. The effect of morphine on CBF was blocked by lidocaine administered simultaneously (Lid + Mor). Readings were made at 15 min after morphine injection. $^{**}P < 0.01$.

administration. Injection of 10 mg/kg morphine (i.p.) did not alter mean arterial blood pressure and heart rate immediately, while significant decreases in cerebral blood flow were noted 10–15 min after administration of this dose of morphine in control and sham-operated animals ($P < 0.05$) (Fig. 3). This figure shows that the decrease in regional blood flow in dependent groups was significantly ($P < 0.05$) less than that in the control and sham -operated groups, but there were no drug-by-time interactions, generally [$F(6,48) = 0.79,\ P > 0.05$].

CBF increased in the frontoparietal cortex ($81.7 \pm 7.2\%$) ($P < 0.001$) 15 min after subcutaneous administration of naloxone (0.5 mg/kg) to morphine-dependent rats (Fig. 4). This elevation was significantly ($P < 0.001$) higher in dependent group than in control or sham-operated groups. Fig. 5 is a tracing of original LDF and arterial blood pressure data collected over 35 min after naloxone injection in dependent rats. The LDF signal was increased during the withdrawal syndrome and returned to baseline approximately 40 min after naloxone injection.

There was no significant difference in regional cerebral blood flow before and after lidocaine administration. Fig. 6 and Fig. 7 indicate that lidocaine significantly reduced the

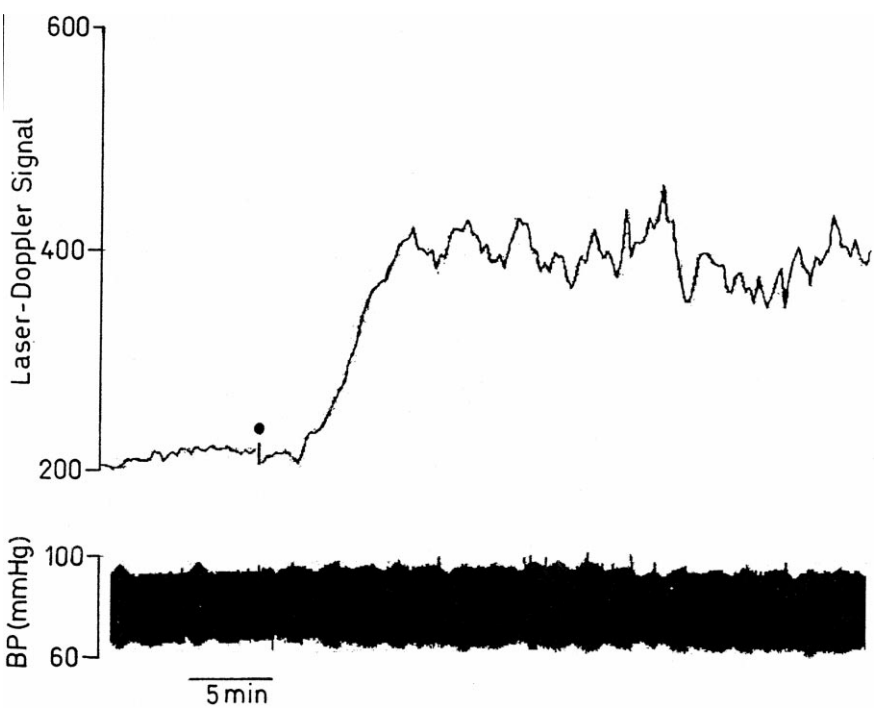

Fig. 5. Original LDF data tracing collected during withdrawal syndrome in dependent rats. Blood pressure remained constant during the experiment. Solid dot represents naloxone injection time.

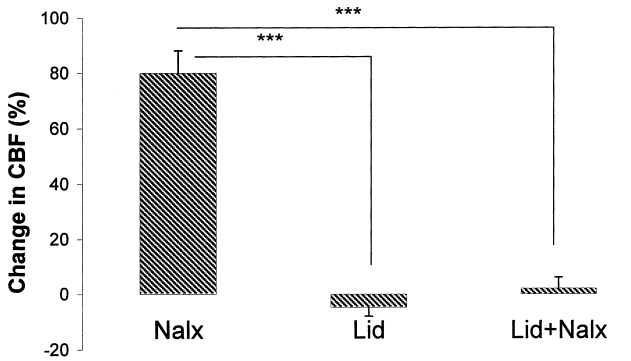

Fig. 7. Effect of lidocaine (Lid, local) on rCBF during naloxone-precipitated withdrawal in dependent rats. Values are means ± S.E.M. ($n = 5$). Response to naloxone (Nal) was significantly reduced when lidocaine was administered simultaneously (Lid + Nal). Readings were made at 15 min after naloxone injection (SC). $^{***}P < 0.001$.

effects of morphine and naloxone on regional blood flow. Injection of morphine (20 mg/kg; i.p.) significantly decreased regional blood flow in naive rats ($P < 0.01$) but lidocaine application inhibited this effect [$F(2,12) = 13.8$, $P = 0.0008$]. In morphine-dependent rats, lidocaine inhibited CBF enhancement during morphine withdrawal [$F(2,12) = 80.55$, $P < 0.0001$].

4. Discussion

The present findings suggest the occurrence of morphine tolerance and dependence in the brain of chronically morphinized rats, as manifested by changes in regional cerebral blood flow (rCBF). In this study, we used LDF to measure rCBF in animals with morphine dependence during naloxone administration, which precipitated the morphine withdrawal. The main finding of this study was an increase in cerebral cortical blood flow after the administration of naloxone, which precipitated the morphine withdrawal syndrome in morphine-dependent rats. The changes in cerebral blood flow were highly reproducible.

Acute morphine treatment (10 mg/kg, i.p.) decreased rCBF in naive rats, but was ineffective in the chronically morphinized rats. It seems likely that the metabolic effects of opiate receptor activation determine the influence of opioids on CBF. Opiates are known to inhibit the release of acetycholine, norepinephrine, substance P, and dopamine in the central nervous system, an effect which may be expected to decrease metabolism (Hoaner et al., 1993). Opioids may reduce CBF via diminishing cerebral metabolic rates of oxygen and/or glucose (Benyo and Wahl, 1996).

Our study showed that naloxone (0.5 mg/kg) increased rCBF in morphine-dependent rats. Previously, Wooten et al. (1982) had found that structures including the central nucleus of the amygdala, several midline anterior thalamic nuclei and the lateral habenular nucleus showed changes in their metabolism during withdrawal. All the structures that are metabolically active during morphine withdrawal are closely connected synaptically. Therefore, many structures may be activated as the result of an increased metabolism in other structures through synaptic connections. As Wooten et al. (1982) reported, glucose utilization in morphine-dependent rats was decreased in frontal cortex and anterior ventral thalamus. They suggested that a return toward the control level occurred during withdrawal. In order to test this possibility, absolute cerebral blood flow must be measured in dependent rats.

Kimes and London (1989) observed no changes in glucose utilization in primary and secondary somatosensory cortices and hindlimb regions mediating somatosensory input during naloxone-precipitated morphine withdrawal, 45 min after pellet removal. However naloxone-precipitated morphine withdrawal produced hypermetabolism in thalamic nuclei. The thalamic areas in which the greatest increases in glucose utilization occurred during naloxone-precipitated morphine withdrawal, were also those in which the greatest decreases were observed after acute morphine administration (Fanelli et al., 1987) consistent with the rebound hypothesis of opioid withdrawal (Wikler, 1980). Hypermetabolism in thalamic nuclei that are involved in somatosensory processing may contribute to the irritability associated with naloxone-precipitated morphine withdrawal (Jaffe, 1985). Nevertheless, the inconsistency that seems to exists between our findings and those of London et al. (1986) could be due to methodological approaches. It may also have resulted from differences in the timing of measurements.

An alternative approach is that increases in metabolism during morphine withdrawal may be activated independently and directly by opiate agonists and antagonists. However, considering the low density of opiate receptor binding sites in the somatosensory cortex, the latter hypothesis is not confirmed. Wooten et al. (1982) reported that there is no clear correlation between those structures that are particularly active during morphine withdrawal and opiate receptor distribution. While the somatosensory cortex is not important in the occurrence of morphine withdrawal, the changes in cerebral blood flow in it may be used as an index for opiate withdrawal.

The mechanisms underlying rCBF changes in opiate dependence are complex and still little understood. Previously, investigators have shown that a higher dose of naloxone increases CBF and cerebral metabolism in rats and dogs (Phillis et al., 1985; Turner et al., 1984) but we used a lower dose which had no effect on CBF of naive rats.

Fig. 6 shows that local application of lidocaine inhibited the effects of morphine on CBF. The mechanism responsible for elimination of the morphine-induced decrease is still unclear. Previously, excitatory and inhibitory effects of opioids had been found after central and local application of morphine (Benyo and Wahl, 1996; Hoaner et al.,

1993; Zieglgansbergar et al., 1976) that may explain the sometimes transient and regionally restricted increase or decrease of CBF. It is widely accepted that increases in neuronal electrical activity are accompanied by increases in local cerebral blood flow and regional metabolic activity in the homonymous activated region (Sokoloff, 1981; Gerrits et al., 1998). The local application of lidocaine also blunted rCBF responses to naloxone. It seems that this elimination may be caused by damping of the presumed underlying heightened cerebral metabolic activity. This explanation needs to be supported by the results of other experiments involving metabolic or electrophysiological measurements. If these findings are confirmed, they may support the metabolic hypothesis of local blood flow control that states that blood flow is tightly coupled to local neuronal functional and metabolic activity (Ngai et al., 1995; Sokoloff, 1981).

Since lidocaine blunted the rCBF responses to both naloxone and morphine, an alternative plausible explanation for effect of lidocaine is that it may affect directly the local cerebral vasculature and is making it unresponsive, independent of any underlying changes in cerebral metabolic activity. There is a wealth of experimental data concerning the effects of lidocaine on the local cerebral vasculature and metabolism (Muir and Ellis, 1995; Altura and Lassoff, 1981). Altura and Lassoff showed that perivascular application of lidocaine to the pial terminal arterioles exerts dose-dependent potent vasodilator actions on these important resistance vessels. In our experiments, we used a lower dose which had no effect on CBF.

In conclusion, the present results are consistent with previous findings of pathological CBF in opiate withdrawal. This study may also provide a framework to study the cellular and molecular mechanisms responsible for coupling neuronal electrical activity with regional alterations in blood flow during precipitation of morphine withdrawal.

References

Altura, B.M., Lassoff, S., 1981. Perivascular action of the local anaesthetic, lidocaine, on pial terminal arterioles: direct observations on the microcirculation. Br. J. Pharmacol. 73, 557–579.

Arbit, E., DiResta, G.R., Galicich, J.H., 1989. Intraoperative measurement of cerebral and tumor blood flow with Laser–Doppler flowmetry. Neurosurgery 24, 166–170.

Badavi, A.A., Evans, C.M., 1982. Production of tolerance and physical dependence in the rat by simple administration of morphine in drinking water. Br. J. Pharmacol. 75, 485–491.

Benyo, Z., Wahl, M., 1996. Opiate receptor-mediated mechanisms in the regulation of cerebral blood flow. Cereb. Brain Metab. Rev. 8, 326–357.

Cohen, S.R., Kimes, A.S., London, D., 1991. Morphine decreases cerebral glucose utilization in limbic and forebrain regions while pain has no effect. Neuropharmacology 30, 125–134.

Fanelli, R.J., Jasinski, D.R., London, E.D., 1987. Differential effects of mu and kappa opioid analgesics on cerebral glucose utilization in the rat. Brain Res. 422, 257–266.

Fukuda, O., Endo, S., Kuwayama, N., Harada, J., Takaku, A., 1995. The characteristics of Laser–Doppler flowmetry for the measurement of regional cerebral blood flow. Neurosurgery 36, 358–364.

Gerrits, R.J., Stein, E.A., Greene, A.S., 1998. Blood flow increases linearly in rat somatosensory cortex with increased whisker movement frequency. Brain Res. 783, 151–157.

Haghparast, A.S., Semnanian, S., Fathollahi, Y., 1998. Morphine tolerance and dependence in the nucleus paragigantocellularis: single unit recording study in vivo. Brain Res. 814, 71–77.

Hauerberg, J., Juhler, M., 1997. Effects of morphine and naloxone on cerebral blood flow and metabolism in experimental subarachnoid hemorrhage. Acta Neurol. Scand. 96, 187–193.

Hoaner, P.J., Whitson, J.T., Kirch, J.R., Traystman, R.J., 1993. Effect of intracarotid and intraventricular morphine on regional cerebral blood flow and metabolism in pentobarbital-anesthetized dogs. Anesth. Analg. 76, 266–273.

Hsieh, J.C., Belfrage, M., Stone, S., Hansson, P., Ingvar, M., 1995. Central representation of chronic ongoing neuropathic pain studies by positron emission tomography. Pain 63 (2), 225–236.

Jaffe, J., 1985. Drug addiction and drug abuse. In: Gilman, A.G., Goodman, L.S., Rall, T.W., Murad, F. (Eds.), Pharmacological Basis of Therapeutics. Macmillan, New York, pp. 532–581.

Kimes, A.S., London, E.D., 1989. Glucose utilization in the rat brain during chronic morphine treatment and naloxone-precipitated morphine withdrawal. J. Pharmacol. Exp. Ther. 248, 538–543.

Krystal, J.H., Woods, S.W., Kosten, T.R., Rosen, M.I., Seibyl, J.P., van-Dyck, C.C., Price, L.H., Zubal, I.G., Hoffer, P.B., Charney, D.S., 1995. Opiate dependence and withdrawal. Am. J. Drug Alcohol Abuse 21, 47–63.

London, E.D., Wilkerson, G., Goldberg, S.R., Risner, M.E., 1986. Effects of l-cocaine on local cerebral glucose utilization in the rat. Neurosci. Lett. 68, 73–78.

Martin, J.H., 1991. Autoradiographic estimation of the extent of reversible inactivaation produced by microinjection of lidocaine and muscimol in the rat. Neurosci. Lett. 127, 160–164.

Muir, J.K., Ellis, E.F., 1995. Acute cocaine administration alters posttraumatic blood pressure and cerebral blood flow in rats. Am. J. Physiol. 268, H68–H73.

Ngai, A.C., Meno, J.R., Winn, H.R., 1995. L-NNA suppresses cerebrovascular response and evoked potentials during somatosensory stimulation in rats. Am. J. Physiol. 269, H1803–H1810.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. 2nd edn. Academic Press, New York.

Phillis, J., DeLong, R.E., Towner, J.K., 1985. Naloxone enhances cerebral reactive hyperemia in the rat. Neurosurgery 17, 596–599.

Rose, J.S., Branchey, M., Buydens-Branchey, L., Stapleton, J.M., Chasten, K., Werrell, A., Maayan, M.L., 1996. Cerebral perfution in early and late opiate withdrawal. Psychiatr. Res. 67, 39–47.

Sokoloff, L., 1981. Relationships among local functional activity, energy metabolism, and blood flow in the central nervous system. Fed. Proc. 40, 2311–2316.

Turner, D.M., Kassell, N.F., Sasaki, T., Comair, Y.G., Boarini, D.J., Beck, D.O., 1984. Effects of naloxone on cerebral blood flow and metabolism in isoflurane/nitrous oxide-anesthetized dogs. Neurosurgery 14, 688–696.

Wikler, A., 1980. Opioid Dependence: Mechanisms and Treatment. Plenum, New York, pp. 141–166.

Wooten, G.F., Distefano, P., Collins, R.C., 1982. Regional cerebral glucose utilization during morphine withdrawal in the rat. Proc. Natl. Acad. Sci. U. S. A. 79, 3360–3364.

Zieglgansbergar, W., Fry, J.P., Herz, A., Moroder, L., Wunsch, E., 1976. Enkephalin induced inhibition of cortical neurones and the lack of this effect in morphine tolerant/dependent rats. Brain Res. 115, 160–164.