Brain Research, 368 (1986) 170–173
Elsevier

BRE 21422

The effect of analgesic doses of morphine on regional cerebral glucose metabolism in pain-related structures

ROBERT M. LEVY, HOWARD L. FIELDS, MICHAEL P. STRYKER and MARY M. HEINRICHER

Departments of Neurological Surgery, Physiology and Neurology, School of Medicine, University of California, San Francisco, California (U.S.A.)

(Accepted November 12th, 1985)

Key words: morphine — analgesia — regional cerebral metabolism — [^{14}C]2-deoxyglucose autoradiography — pain

The effect of morphine on regional cerebral glucose metabolism was measured in rats using high resolution [^{14}C]2-deoxyglucose autoradiography with concurrent confirmation of morphine-induced analgesia measured by tail-flick latency to noxious heat. Within the limits of resolution of this technique, doses of morphine sufficient to inhibit the tail-flick reflex had no significant effect on glucose metabolism in structures implicated in the modulation of pain.

While the antinociceptive effect of morphine is well-known, a direct effect of this opiate on glucose metabolism in regions of the central nervous system implicated in pain modulation has not been well-characterized. While the effects of morphine on regional cerebral glucose metabolism (RCGM) have been reported[9,11], behavioral testing was not used to confirm morphine analgesia. Results obtained for RCGM during activation of pain-modulating systems by electrical stimulation of the periaqueductal gray may not be comparable with the effects of systemically administered morphine[4]. In the experiments reported here, we used tail-flick (TF) latency to noxious heat in rats to document morphine analgesia concurrently with measurement of RCGM in pain-related structures.

Fourteen adult male Sprague–Dawley rats were anesthetized with pentobarbital (50 mg/kg, ip) and jugular venous and femoral arterial and venous catheters were placed. Body temperature was maintained at 38 °C with a circulating water pad. Rats were maintained in a lightly anesthetized state using a continuous infusion of methohexital (15–30 mg/kg/h, iv), which prevented spontaneous movement and signs of discomfort but allowed TF responses at stable latencies. TF latency was tested by focusing a projector lamp onto the blackened ventral surface of the rat's tail. A thermistor probe placed in contact with the tail provided the signal for feedback control of the heat stimulus. TF usually occurred 3–4 s after the onset of heat. If no TF occurred, the heat was turned off at 10 s to prevent tissue damage.

After stable TF latencies were obtained, either morphine sulfate (MS) (5 mg/kg, iv) or saline was administered; Levine et al.[10] have shown that this dose of morphine induces profound analgesia without significant sedation. TF testing was repeated 2 min after administration of MS or saline; in all rats injected with MS, TF increased to cutoff. [^{14}C]2-deoxyglucose (100 μCi/kg, 56 mCi/mmol, Amersham CFA562, iv) was then administered by continuous infusion over 15 min. Isotope was not administered by bolus injection[13] because of evidence[2,6] that some morphine-sensitive cells in the rostral ventral medulla undergo spontaneous cycles of activity that last up to several minutes. Infusion for an extended period produced a prolonged period of availability of tracer in plasma; therefore, values of RCGM should reflect glucose utilization averaged over several cycle times for these cells. Arterial blood samples were obtained

Correspondence: R.M. Levy, c/o The Editorial Office, Department of Neurological Surgery, 1360 Ninth Avenue, Suite 210, San Francisco, CA 94122, U.S.A.

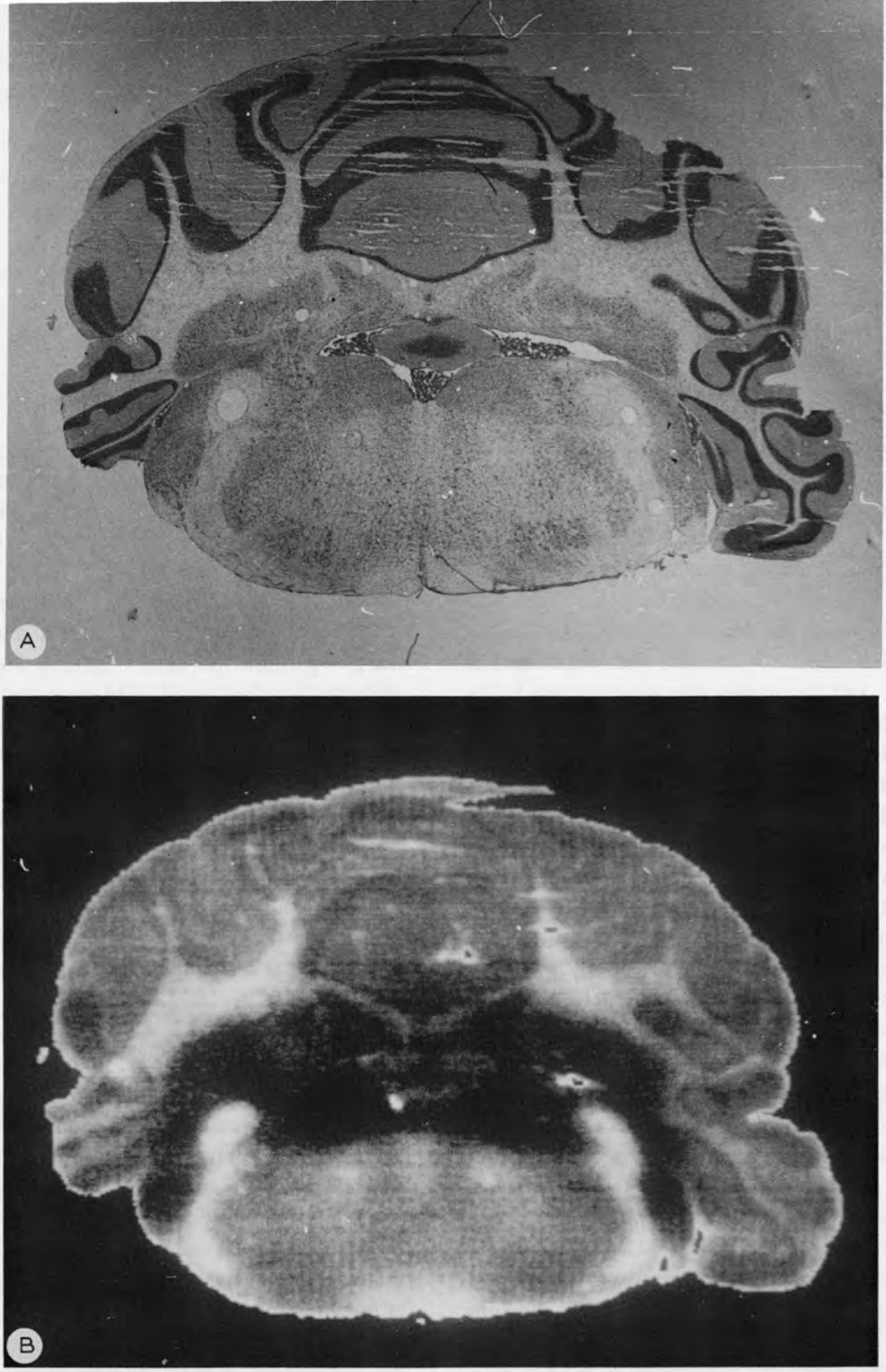

Fig. 1. A: Nissl-stained section taken through the rostral rat medulla that shows the nucleus paragigantocellularis and nucleus raphe magnus. B: [^{14}C]2-deoxyglucose autoradiograph of the section shown in A. Despite the high resolution of this technique, no focal areas of increased [^{14}C]2-deoxyglucose uptake are seen after morphine administration.

at regular intervals to assess plasma glucose and isotope concentrations.

TF latency was tested 35 min after infusion of isotope was completed. Heating for 10 s did not cause a TF response in any MS-treated rat. TF latency remained at baseline in saline-treated rats, however. The opiate antagonist naloxone (2 mg/kg, iv) was administered to confirm that the lack of a TF in MS-treated rats was caused by administration of morphine; as a control, naloxone also was administered to saline-treated rats. In all but two rats, which were discarded from analysis, TF latency returned to baseline after administration of naloxone.

Rats were sacrificed 50 min after beginning [^{14}C]-2-deoxyglucose infusion, immediately after naloxone testing was completed. Brains were removed, frozen in liquid freon (−75 °C) and then 20-μm sections were cut at −20 °C, dried onto cover glasses and exposed to Kodak SB5 film using standard autoradiographic techniques[1]. Tissue on autoradiographic slides was then stained using the Nissl technique. Autoradiographs and Nissl-stained sections were digitalized at 20 μm/pixel and the images were superimposed. Regions of interest on the Nissl sections were delineated using a bit pad cursor and were used to calculate RCGM for identical areas on the autoradiographic sections using methods described by Sokoloff et al.[13].

Values for RCGM for CNS regions thought to be involved in pain modulation and for control regions, expressed relative to RCGM values for cerebellum, are listed in Table I. The cerebellum was used as a reference region because it is an essentially opiate-insensitive structure. Control values of RCGM found in these experiments are comparable to values reported by others for anesthetized rats[13]. The rate of glucose metabolism for each region is expressed relative to the rate for the cerebellum to correct for nonspecific, non-analgesia-related effects of morphine. The slight decreases in values for RCGM in most structures after morphine administration, which suggests that morphine suppresses RCGM diffusely, are not statistically different from control values for the respective regions. Because arterial PCO_2 was not measured, we cannot exclude the possibility that the low analgesic dose of MS used in this study induced a mild hypercarbia that might have masked a positive result.

These findings are consistent with the results of a

TABLE I

The effect of morphine on regional cerebral glucose metabolism

Values are expressed as ($RCGM_{region}/RCGM_{cerebellum}$) × 100 and S.E.M.

Region	*Control*	*Morphine*
N. reticularis paragigantocellularis lateralis	93.8 ± 3.7	86.4 ± 1.9
N. raphe magnus	87.6 ± 3.5	81.9 ± 2.6
N. reticularis gigantocellularis	88.6 ± 3.8	82.8 ± 1.8
Facial nerve nucleus	97.6 ± 5.1	91.0 ± 3.1
Trigeminal nerve nucleus	90.0 ± 3.8	85.0 ± 1.3
Pyramidal tract	59.4 ± 3.4	62.2 ± 2.7
Superior olive	136.6 ± 10.6	129.9 ± 6.1
Periaqueductal gray	99.5 ± 5.6	92.0 ± 3.3
Midbrain reticular formation	90.2 ± 4.4	85.1 ± 2.6
Inferior colliculus	115.1 ± 4.8	107.9 ± 2.2

number of studies that showed acute morphine administration had no specific effect on the RCGM of structures implicated in the mechanisms of morphine analgesia[3,5]. Sakurada et al.[11] found diffuse decreases in RCGM of rats after 5–30 mg/kg of morphine was administered. In more detailed studies, this group[9] found that doses of 1.0 or 2.5 mg/kg of morphine had no effect on RCGM measured 1 h after administration of the opiate. Administration of 5.0 mg/kg of morphine caused changes in RCGM of the substantia nigra, mammillary body and medial division of the lateral habenular nucleus, but had no effect on RCGM overall. Hiesiger et al.[8] measured RCGM 10 min after administration of 5.0 mg/kg of morphine and found that while there was a diffuse decrease in RCGM, a statistically significant reduction in RCGM was found only in the medial thalamus and habenula.

Because the antinociceptive effects of morphine were not documented in animals in which RCGM was measured in any of these studies, it is possible that either profound sedation or a failure to produce analgesia in all morphine-treated animals may have obscured any effect of morphine in pain-related structures. In a study of the effects of electrical stimulation of the periaqueductal gray on RCGM in rats, Beitz and Buggy[4] documented analgesia before determination of RCGM. They found increases in RCGM in nucleus reticularis paragigantocellularis, the ventral portion of the nucleus reticularis gigantocellularis and nucleus cuneiformis, and in the spinal trigeminal nucleus and the substantia gelatinosa. Their data are difficult to evaluate, however, and

electrical stimulation may have had a non-specific effect on RCGM. Moreover, the effects of morphine and electrical stimulation of the periaqueductal gray on RCGM may not be comparable. In the experiments reported here, RCGM was measured during behaviorally documented morphine-induced analgesia. We found no statistically significant changes in RCGM in the midbrain periaqueductal gray, the nucleus raphe magnus, or in the nucleus reticularis paragigantocellularis.

It was shown recently that different classes of cells in the rostral ventral medulla in the region of the nucleus raphe magnus have predictable and differential changes in spontaneous discharge and, presumably, metabolic rate after administration of morphine systemically[2,6]. Although these populations of cells in the rostral ventral medulla are either activated or inhibited by systemic morphine under the exact conditions of this experiment[5], we observed no changes in RCGM in this region after morphine administration.

Values for RCGM reported here were measured for regions delineated on Nissl-stained sections; there was no obvious anatomical compartmentalization with respect to the effects of morphine on neuronal activity[2,6]. Moreover, direct measurement of RCGM from the autoradiographs provided no evidence that morphine altered metabolic activity in small, discrete regions of the rostroventral medulla. Electrophysiologic experiments[2,6] have not determined whether neurons excited by morphine are concentrated in areas that are larger than the limit of resolution of our autoradiographic technique, which is calculated to be greater than 100 μm[7]. Thus, regions in which RCGM is increased after administration of morphine may be so small that they were not detected with this technique. It is also not clear whether the metabolic activity of these neurons, measured at their somata, is closely coupled to their discharge frequency; close coupling may be the rule in cerebral cortex[12], but there appear to be some exceptions for brainstem neurons[1].

It is also probable that cells in the rostral ventral medulla that are either activated or inhibited by morphine administration are not spatially segregated; thus, despite profound effects on individual morphine-sensitive neurons, there may be no overall effect on RCGM. Results of our previous studies[6,9] suggest that it is unlikely that the population of morphine-sensitive cells in the rostral ventral medulla is so small that significant stimulation of these cells is not sufficient to alter the overall RCGM in these areas.

R.M.L. is supported in part by a fellowship from the Research Foundation of the American Association of Neurological Surgeons, H.L.F. by PSH and DA 01949, and M.M.H. by PHS Grant 5T2NS07067. We thank Neil Buckley for editing the manuscript.

1 Auker, C.R., Meszler, R.M. and Carpenter, D.O., Apparent discrepancy between single-unit activity and [^{14}C]deoxyglucose-labeling in optic tectum of the rattlesnake, *J. Neurophysiol.*, 49 (1983) 1504–1576.
2 Barbaro, N., Heinricher, M.M. and Fields, H.L., Putative pain-modulating neurons in the rostral ventral medulla: reflex-related activity predicts effects of morphine, *Brain Research*, in press.
3 Basbaum, A.I. and Fields, H.L., Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry, *Annu. Rev. Neurosci.*, 7 (1984) 309–338.
4 Beitz, A.J. and Buggy, J., Brain functional activity during PAG stimulation-produced analgesia: a 2-DG study, *Brain Res. Bull.*, 6 (1981) 487–494.
5 Fields, H.L. and Basbaum, A.I., Brainstem control of spinal pain-transmission neurons, *Annu. Rev. Physiol.*, 40 (1978) 217–248.
6 Fields, H.L., Vanegas, H., Hentall, I.D. and Zorman, G., Evidence that disinhibition of brainstem neurones contributes to morphine analgesia, *Nature (London)*, 306 (1983) 684–686.
7 Goochee, C., Rasband, W. and Sokoloff, L., Computerized densitometry and color coding of [^{14}C]deoxyglucose autoradiographs, *Ann. Neurol.*, 7 (1980) 359–370.
8 Hiesiger, E.M., Voorhies, R.M., Lipschutz, L., Basler, G., Shapiro, W.R. and Pasternak, G.W., The effects of morphine on glucose metabolism ([^{14}C]2-deoxyglucose) in rat brain as measured by quantitative autoradiography, *Neurosci. Abstr.*, 1 (1983) 138.
9 Ito, M., Suda, S., Namba, H., Sokoloff, L. and Kennedy, C., Effects of acute morphine administration on local cerebral glucose utilization in the rat, *J. Cereb. Blood Flow Metab.*, 3 (1983) S574–S575.
10 Levine, J.D., Murphy, D.T., Seidenwurm, D., Cortez, A. and Fields, H.L., A study of the quantal (all-or-none) change in reflex latency produced by opiate analgesics, *Brain Research*, 201 (1980) 129–141.
11 Sakurada, O., Shinohara, M., Klee, W.A., Kennedy, C. and Sokoloff, L., Local cerebral glucose utilization following acute or chronic morphine administration and withdrawal, *Neurosci. Abstr.*, 2 (1976) 613.
12 Schoppmann, A. and Stryker, M.P., Physiological evidence that the 2-deoxyglucose method reveals orientation columns in cat visual cortex, *Nature (London)*, 293 (1981) 574–576.
13 Sokoloff, L., Reivich, M., Kennedy, C., DesRosiers, M.H., Patlak, C.S., Pettigrew, K.D., Sakurada, O. and Shinohara, M., 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 (1977) 897–916.