

ANTERIOR CEREBELLUM AS A SITE FOR MORPHINE ANALGESIA AND POST-STIMULATION ANALGESIA

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Summary : The microinjection of 10 μ g Morphine into culmen region of anterior cerebellum produced profound analgesia in rats, and this was antagonised with intraperitoneal administration of naloxone. On the other hand, the same injection of morphine into lobus simplex and declive region of posterior cerebellum was without any effect on nociception. Further it was observed that chronic surgical ablation of culmen-centralis region of anterior cerebellum markedly diminished the duration of analgesia elicited with systemic administration of morphine, though ablation *per se* had no influence on nociception. Also, the focal electrical stimulation of culmen region for a brief period exhibited post-stimulation analgesia. These findings indicate that anterior cerebellum specifically plays some role in the modulation of physiological mechanisms of pain relief.

Key words : morphine analgesia cerebellum post-stimulation analgesia naloxone

INTRODUCTION

There is now considerable evidence that morphine analgesia can be elicited following its injection into the different loci of the brain: the thalamus (2); the hypothalamus (4, 7, 19, 20); the periaqueductal gray matter (8, 14, 16); the floor of the fourth ventricle (6) and the ventral surface of the brainstem (3, 15). It has also been demonstrated that focal electrical stimulation of some of the aforementioned sites such as, PAG (10), hypothalamus (1) and thalamus (11) can result in the development of antinociceptive response in rats. Siegel and Wepsic (17) reported that antinociceptive response can also be elicited following focal electrical stimulation of anterior cerebellum in monkeys. In the present

paper, we now report that the microinjection of morphine in anterior cerebellum produces profound analgesia in rats, and that the analgesic response of morphine after its systemic administration is greatly attenuated in chronic anterior cerebellectomised rats. We further observed that focal electrical stimulation of anterior cerebellum in rats produces analgesia confirming the observation of Siegel and Wepsic (17) in monkeys.

MATERIALS AND METHODS

The experiments were carried out on male albino rats (200–250 g, CF strain). The surgical procedures for implantation of guide cannula or electrodes as well as for partial cerebellectomy were carried out under pentobarbitone-Na (40 mg/kg, ip) anaesthesia in aseptic conditions.

Injection of morphine into cerebellum: A stainless steel guide cannula (22 G) with a stylet inserted into its shaft of 1 mm length was chronically implanted onto the cerebellar surface under stereotaxic guidance. After a week, the analgesic test (15) was carried out following infusion of morphine solution through the implanted cannula in a volume of 1 μ l by a microinfusion pump which delivered this volume from a 10 μ l Hamilton syringe in 30 sec. For such infusion of drug solution, the stylet was removed and a hollow stainless steel needle (28 G) was inserted which was already connected with Hamilton Syringe by a length of polythene tubing filled with drug solution to be injected. The infusion needle was inserted 1 to 2 mm deep from the tip of the guide cannula. For injection of morphine into the anterior cerebellar region, or into posterior cerebellar cne, the guide cannula was implanted in the midline 2.5 to 3.0 mm or 3.0 to 4.0 mm posterior to Lambda respectively.

At the end of each experiment, the postmortem examination was made for confirmation of the position of implanted cannula as well as to note the spread of morphine solution into the cerebellar tissue. For this purpose, the animal was anaesthetised with pentobarbitone-Na, and Evans blue dye (2%) was infused following the same procedure as that for morphine. After 5 min, the brain was perfused with 100 ml buffered 10% formalin-saline and the whole brain was removed, and the location of the guide cannula and spread of dye into the tissue were noted through magnifying lens, following an incision of the cerebellum in sagittal plane below the tip of guide cannula.

Partial cerebellectomy: The head of the anaesthetised animal was fixed in the stereotaxic apparatus. The skull bone was exposed by giving a midline incision on the skin. A burr hole was made in the midline on the skull

either 2.5 mm or 3.5 mm posterior to lambda and then either the anterior cerebellar region or posterior cerebellar one was partially removed respectively by suction with an aspirator which was connected with a glass capillary tube inserted through the burr hole.

After a week, the analgesic tests were carried out before and after intraperitoneal administration of morphine (10 mg/kg).

At the end of the experiment, the postmortem study was done for the confirmation of the extent of cerebellar area removed. The animal was anaesthetised with pentobarbitone-Na and its head was fixed in stereotaxic apparatus. The brain was perfused with 100 ml buffered 10% formalin-saline solution. After 10 min, the skull bone was removed carefully and the depth and the extent of the cerebellar area/areas ablated was determined and plotted on a stereotaxic map of cerebellum.

Electrical stimulation of anterior cerebellum : The animal's head under anaesthesia was fixed in the stereotaxic instrument. One bipolar electrode made of twisted stainless steel wire (0.2 mm diameter) teflon coated except for the cross-sectional area at the tip was implanted in the midline 2 to 2.5 mm posterior to lamda, and 0.5 to 1 mm deep from the dura so that the tip lay in the culmen area. The electrode and connector were secured to the skull by flowing dental acrylic cement around them and two stainless steel screws threaded into the skull. After a week, the analgesic test was carried out following electrical stimulation of cerebellar tissue through implanted electrode. The square wave electrical pulses were delivered from Grass S88 stimulator (USA).

At the end of the experiment, the animal was anaesthetised with pentobarbitone and the brain was perfused with buffered formalin-saline solution with the electrode *in situ* position. Serial frozen sections were cut at 40 μ m and alternate sections stained with Thionin for histological verification of location of electrode placements.

RESULTS

Morphine infusion into cerebellum : The results show that a dose of morphine as small as 10 μ g infused into anterior cerebellar region produced profound analgesia in 6 rats (Fig. 1-A). In all these rats, the analgesia began within 3 min of morphine infusion with the peak analgesia reached around 30 min. The analgesia lasted for more

than 60 min. The postmortem examination in these animals showed that morphine solution was distributed mainly into the culmen region in 4 rats and partially into dorsal lobus centralis in two rats. It has further been observed that the analgesia elicited from the above anterior cerebellar region was antagonised following naloxone administration. The results are shown in Fig. 1-B Thus in two rats, in which the analgesia began to develop following morphine injection ($10 \mu\text{g}$) into culmen of anterior cerebellum,

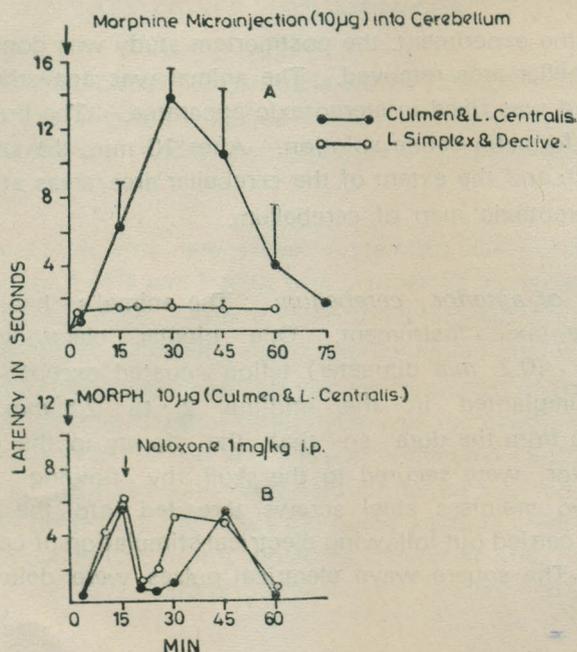


Fig. 1-A : Shows analgesia following microinjection of morphine ($10 \mu\text{g}$) into culmen-centralis region of anterior cerebellum ($n=6$). Analgesia did not occur following injection into L. simplex and declive region of posterior cerebellum ($n=4$).

B : Shows that naloxone (1 mg/kg , ip) antagonises analgesia elicited following injection of $10 \mu\text{g}$ morphine into culmen-region ($n=2$). Naloxone was administered 15 min after morphine injection.

the intraperitoneal administration of 1 mg/kg naloxone almost immediately antagonised the analgesia (Fig. 1-B).

In four animals, the microinfusion of morphine ($10 \mu\text{g}$) did not produce analgesia. The postmortem examination showed that in two rats, the cannula was implanted in

between the inferior colliculus and cerebellum, and in other two rats, the anterior cerebellar tissue showed inflammatory reactions around the implanted cannula.

The analgesic response of morphine did not occur following microinfusion of same dose of morphine into posterior cerebellar regions such as lobus simplex and declive in 4 rats (Fig. 1-A).

Morphine analgesia in partial cerebellectomized animals:

The results in Fig. 2 show that removal of anterior cerebellar region in 5 rats exerted a remarkable influence on analgesic response that occurred following i. p.

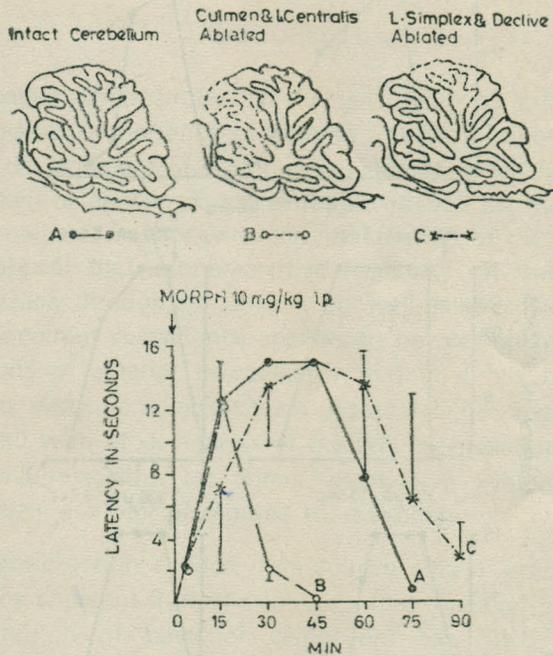


Fig. 2: Shows the analgesic response following i. p. administration of morphine (10 mg/kg) in intact control (n=5), culmen and centralis ablated (n=5), and lobus simplex and declive ablated (n=5) groups of rats. A marked diminution of duration of analgesia occurred in culmen centralis ablated group.

injection of morphine (10 mg/kg). Thus, following morphine injection in control animals (n=5), the analgesia generally reached to its peak response within 15 min and the duration of analgesia lasted for about 75 min. On the other hand, the i.p injection of same dose of morphine in chronic anterior cerebellectomised animal produced analgesia but the

duration of analgesia was greatly shortened. Thus the analgesia remained for not more than 45 min as compared to about 75 min in control animals.

The postmortem examination in these animals showed that this marked diminution of analgesic response of morphine was associated with the ablation of culmen or the culmen and dorsal lobule of lobus centralis together. It was interesting to observe that cerebellar ablation *per se* did not modify the nociceptive response of the animal.

Such attenuation of morphine analgesia was not observed following ablation of posterior cerebellar region (lobus simplex and declive) in 5 rats. Rather, a tendency for prolongation of analgesia was apparent (Fig. 2).

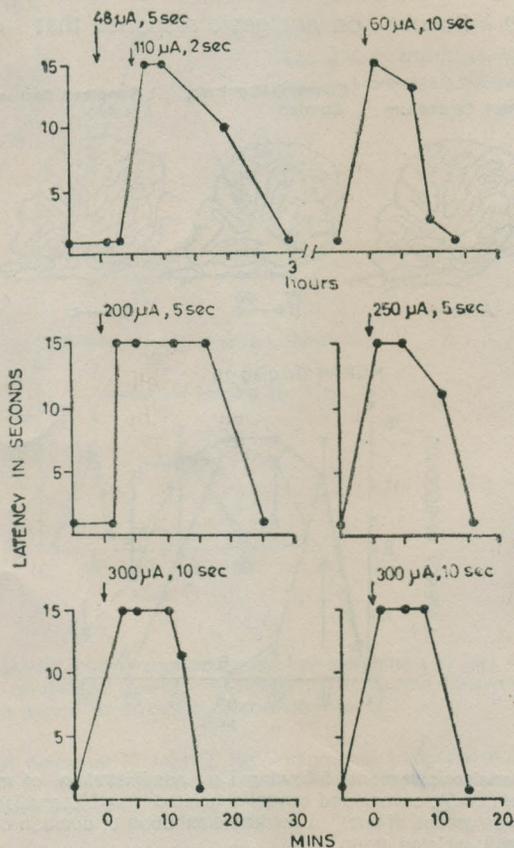


Fig. 3 : Shows post-stimulation analgesia in 5 individual rats following bipolar electrical stimulation (pulse width 0.2 msec, 70Hz) of anterior cerebellum. Arrow indicates application of current for particular duration.

Top : shows analgesia in one rat with 1st and 2nd stimulation at an interval of 3 hrs between two stimulations. Electrode was located within the culmen.

Middle : shows analgesia in two rats. Electrode was located at the cortical surface of culmen region.

Bottom : shows analgesia in two rats with electrode located in lobus centralis but close to culmen region.

Focal electrical stimulation of anterior cerebellum:

Following focal electrical stimulation (0.2 msec pulse-width; 70 Hz) of culmen region for 5 to 10 sec, in 5 animals, the post-stimulation analgesia developed (Fig. 3). But the current strength and the duration of post-stimulation analgesia showed variation from animal to animal, which was found to be related with the location of the electrode. Thus in two rats with electrode located within culmen cortical region, a current strength between 90 and 110 μA produced analgesia lasting for about 5-15 min. One of the rats was again tested 3 hr after first stimulation experiment for analgesia with 2nd electrical stimulation. The result is shown in Fig. 3 (top trace). Analgesia occurred again with a current of 60 μA applied for 10 sec.

DISCUSSION

The present results demonstrate that culmen region of anterior cerebellum in rats bears certain physiological importance in relation to the antinociceptive response of morphine. Thus the development of prolonged analgesia following direct micro-injection of morphine into culmen or culmen-dorsal centralis, as well as, the marked diminution of duration of morphine analgesia in rats with chronic ablation of the same area, if considered together, it would appear that antinociceptive response of systemically administered morphine although occurs through its action on well-established analgesic sites in the brain stem, but its concurrent sustainance appears to be dependent on the action of morphine on target regions of anterior cerebellum. In fact it has been shown (13) that morphine, following its systemic administration in rats, reaches also very rapidly into cerebellar tissue, *parri passu* with other regions of the brain. Thus about 146% of the amount of morphine systemically injected in rat enters cerebellum which is closely similar to values obtained for other anatomical regions of the brain.

That the analgesic action elicited from culmen area of anterior cerebellum appears to be specific becomes apparent from the findings that injection of morphine into lobus simplex or declive region of posterior cerebellum has been found to be ineffective to manifest antinociceptive response. Moreover, Yaksh *et al.* (21) have earlier shown that microinjection of morphine into other regions of rat cerebellum, such as, ventral lobule of centralis, uvula, and into different white matter regions close to lobus centralis, culmen, lobus simplex, uvula, nodulus and lingula failed to elicit analgesia.

It has also been shown in the present experiments, that morphine analgesia elicited from culmen region of anterior cerebellum is completely antagonised by systemic injection of naloxone. This obviously indicates that analgesic action is mediated through opiate

receptors. But the opiate receptor bindings studies in rat brain with 3H-naloxone showed that cerebellum is the poorest binding site for naloxone as compared to that for other opiate binding sites of brainstem region (5). This may seem apparently inconsistent with the present findings of blockade of cerebellar mediated morphine analgesia with naloxone. However, it is to be noted that receptor binding studies have not been explored critically in different anatomical regions of cerebellum, therefore, even the presence of high affinity opiate receptors in culmen region of anterior cerebellum might have been overlooked in such studies, and such argument can also be held true for the report of the presence of small amount of enkephalin in cerebellar cortex (9). Recently, Simon (18) has reported that (3H)-etorphine stereospecifically binds in human cerebellar cortex in the range of 0.12-0.07 $\mu\text{mol}/\text{mg}$ protein and such bindings are also observed in cow, cat, dog and monkey.

Since several workers (1,10,11) have reported that electrical stimulation of certain specific sites in brain stem produces analgesia, and the same sites have already been shown to be sites in which morphine injection elicits marked analgesia, therefore, a few experiments were carried out also to examine analgesia following electrical stimulation of culmen region. The results showed that brief stimulation of culmen for 5 to 10 sec with current varying from 60-300 μA exhibits development of analgesia which persists after withdrawal of stimulus. The duration of post-stimulation analgesia varied between 5 and 10 minutes, and in one case, it remained for 20 minutes. The development of analgesia following electrical stimulation of cerebellum has earlier been reported by Siegel and Wepsic (17). Interestingly, they reported that maximum analgesic response (against noxious electrical shocks in tail) developed in monkeys following electrical stimulation (0.2 mA) of intermediate anterior lobe (culmen) as well as its physiologically related rostral dentate-inter-positus-brachium conjunctivum region. These observations in conjunction with our present finding indicate in general that culmen area of anterior cerebellum can serve as important locus whose activation either with narcotic drug or electrical stimulus can arouse central mechanisms of pain suppression.

It is fairly well-established that there exists a powerful pain suppressive system in brainstem reticular formation (RF) concentrated specially in periventricular, gray, PAG and reticulogiantocellularis area, and its activation diminishes responsiveness to noxious stimuli, atleast in part, through descending spinal pathways which block transmission of nociceptive stimuli through the spinal cord. Since it is well known that connection between RF and cerebellum are extensive, therefore, it is possible that culmen exhibits

its antinociceptive effect via activation of brainstem pain suppression mechanisms. Lastly it is interesting to mention the report of Mehler (12) who observed that in medulla oblongata, the surgical lesion of fibres in retro-olivary levels in two cancer patients (having intractable pain), produced high level of analgesia, but often in some cases, the pain returns after occlusion of the posterior inferior cerebellar artery.

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REFERENCES

1. Balagura, S. and T. Ralph. The analgesic effect of electrical stimulation of the diencephalon and mesencephalon. *Brain Res.*, **60** : 369-379, 1973.
2. Buxbaum, D.M., G.G. Yarbrough and M.E. Carter. Dose-dependent behavioral and analgesic effects produced by microinjections of morphine-sulphate into the anterior thalamic nuclei. *The Pharmacologist*, **12** : 210, 1970.
3. Dey, P.K. and W. Feldberg. Analgesia produced by morphine when acting from the liquor space. *Brit. J. Pharmacol.*, **58** : 383-393, 1976.
4. Foster, R.S., D.J. Jenden and P.A. Lomax. A comparison of the pharmacological effects of morphine and N-methyl-morphine. *J. Pharmac. Expt. Ther.*, **157** : 185-195, 1967.
5. Garcin, F. Opiate receptor binding studies in rat brain during morphine dependence and early abstinence. In: *Neuropsychopharmacology*, Eds. P. Denikar, G. Radouze-Thomas, A. Villeuve, B. Barnonet Lacroix, F. Garcin, Vol. 2, pp. 1295-1305, Pergamon Press, New York, 1978.
6. Herz, A., K. Albus, J. Metys, P. Schubert and H. Teschemacher. On the central sites for the antinociceptive action of morphine and fentanyl. *Neuropharmacology*, **9** : 539, 1975.
7. Jacquet, Y.F. and A. Lajtha. Morphine action at central nervous system in rat : Analgesia or hyperalgesia depending on site and dose. *Science*, **182** : 490-492, 1973.
8. Jacquet, Y.F. and A. Lajtha. Paradoxical effects after micro-injection of morphine in the periaqueductal gray matter in the rat. *Science*, **185** : 1055-1057, 1974.
9. Kobayashi, R., M. Palkovits, R. Miller, K.J. Chang and P. Cuatrecasas. *Life Sci.* **22** : 527-530, 1977.
10. Liebeskind, J.C., G. Guilbaud, J.M. Besson and J.L. Olivers. Analgesia from electrical stimulation of the periaqueductal gray matter in the cat. Behavioral observation and inhibitory effects on spinal cord interneurons. *Brain Res.*, **50** : 441-446, 1973.
11. Mayer, D.J., and J.C. Liebeskind. Pain reduction by focal electrical stimulation of the brain: An anatomical and behavioral analysis. *Brain Res.*, **68** : 73-93, 1974.
12. Mehler, W.R. Central pain and the spinothalamic tract. In: *Advances in Neurology (International Symposium on pain)*. Ed. J.J. Bronica, Vol. 4, pp. 127-146, Raven Press, New York, 1974.

13. Oldendorf, W.H. Factors affecting passage of opiates through the blood-brain barrier. In: Factors affecting the action of narcotics. Eds. M.L. Adler, L. Manara, and R. Samanin, pp. 221, Raven Press, New York, 1978.
14. Pert, A. and T. Yaksh. Sites of morphine induced analgesia in the primate brain: Relation to pain pathways. *Brain Res.*, **80** : 135-140, 1974.
15. Ray, A.K. and P.K. Dey. Morphine analgesia following its infusion into different liquor spaces in rat brain. *Arch. Int. Pharmacodyn.*, **246** : 108-117, 1980.
16. Sharpe, L.C., J.E. Garnett and T.N. Cicere. Analgesia and hyperactivity produced by intracranial microinjections of morphine into the periaqueductal gray matter of the rat. *Behav. Biol.*, **11** : 303-313, 1974.
17. Siegel, P. and J.G. Wepsic. Alteration of nociception by stimulation of cerebellar structures in the monkey. *Physiol. Behav.*, **13** : 189-194, 1974.
18. Simon, E.J. The opiate receptors. In: Receptors in Pharmacology Eds. J.R. Smythies and R. J. Bradley, p. 257, Marcel Dekker Inc, New York, 1978.
19. Tsou, K. and C.S. Jang. Analgesic effect of intraventricular or intracerebral microinjection of morphine. *Acta Physiologica Sinica*, **25** : 119-128, 1962.
20. Tsou, K. and C.S. Jang. Studies on the site of analgesic action of morphine by intracerebral micro-injection. *Sci. Sinica*, 1099-1109, 1964.
21. Yaksh, T.L., J.C. Yeung and T.A. Rudy. Systematic examination in the rat of brain sites sensitive to the direct application of morphine: observation of differential effects within the periaqueductal gray. *Brain Res.*, **114** : 83-103, 1976.