

# MORPHINE HYPERTHERMIA IN RATS : ROLE OF NEUROCHEMICAL SUBSTANCES IN BRAIN

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**Summary :** Central neurochemical mechanism underlying the hyperthermic effect of morphine has been investigated in rats. 200  $\mu$ g morphine hydrochloride, when administered through cerebroventricular route at different seasonal air temperature, caused a rise in rectal temperature of rats. This hyperthermia was not affected by prior administration of antiserotonergic (pCPA, 5,6-DHT) or anticatecholaminergic (PBZ, 6-OHDA) drugs, as well as by PGE synthetase inhibitor, indomethacin. Similarly, cholinergic muscarinic or nicotinic receptor blockers, such as atropine and pentolinium/D-tubocurarine respectively, were ineffective to modify it. Whereas, the depletion of acetylcholine in brain by pretreating the animals with hemicholinium profoundly delayed the hyperthermia, suggesting a central cholinergic involvement in morphine induced hyperthermia in rats.

**Key words :** morphine  
intracerebroventricular infusion

hyperthermia

hemicholinium  
indomethacin

## INTRODUCTION

Several workers (8-10) have reported that acute administration of morphine in rats, results in a biphasic response on their body temperature. In 1968, Paolino and Bernard (17) had first suggested that this biphasic thermal effect of morphine is dependent on ambient temperature. Thus, they observed hypothermia at low ambient temperature ( $5^{\circ}\text{C}$ ) and hyperthermia alone at high ambient temperature ( $32^{\circ}\text{C}$ ) with intracerebral or systemic injection of morphine. The influence of seasonal ambient temperature on morphine-induced alteration in body temperature in rats has also been reported by us (21) and we observed that the cerebroventricular administration of even high dose (200  $\mu$ g) of morphine resulted in hyperthermia alone at high ambient temperature.

Since morphine has been reported to alter the concentration or rate of turnover of several neurotransmitters in the brain including catecholamines, 5-hydroxytryptamine

and acetylcholine (3), it seems plausible that morphine might alter the body temperature by influencing the release or action of these naturally occurring substances in the brain. Recent literatures report their involvement in both hypo and hyperthermic effect of morphine in rats (2, 15, 16). In our previous report (21), we tried to demonstrate the role of catecholaminergic system in morphine-induced hypothermia in rats exposed to low seasonal air temperature.

The present work was aimed to explore the role of central cholinergic, serotonergic or catecholaminergic mechanism in morphine-induced hyperthermia in rats observed at higher ambient temperature.

Prostaglandin of E series being the mediator of pyrogen fever (5, 13), its role if any, in this hyperthermic response of morphine, was also studied.

#### MATERIALS AND METHODS

Experiments were carried out on different groups of male albino rats (CF strain, 150-250 g) reared up at various ambient temperatures (range 17.5° to 34.5°C) existing at different seasons of the year. Drugs (morphine hydrochloride, U.P. Govt. Opium Factory; D-tubocurarine chloride, DL-p-chlorophenylalanine methyl ester hydrochloride, 5,6-dihydroxytryptamine creatinine sulphate, 6-hydroxydopamine hydrobromide and indomethacin, Sigma Chemical Co., U.S.A.; atropine sulphate, Macfarlan Smith Ltd., U.K.; pentolinium tartarate, May and Baker Ltd., India; hemicholinium-3 bromide, obtained from the Department of Pharmacology, Institute of Medical Sciences, B.H.U.) were prepared in 0.9% (W/V) sterile normal saline. Ascorbic acid (1 mg/ml) was added to the solution of 6-OHDA and 5,6-DHT for preventing oxidation of these compounds. Indomethacin was dissolved in saline by adding a pinch of sodium bicarbonate in solution. All the drugs were administered through cerebroventricular route (icv) except indomethacin and p-CPA solutions which were also administered intraperitoneally (ip). Morphine was administered in a fixed dose of 200  $\mu$ g in all the experiments.

The procedures followed for intraventricular administration of drugs and measurement of rectal temperature of rats were same as described previously by us (21). A stainless steel guide cannula was chronically implanted in the lumen of right lateral cerebral ventricle. A constant volume (20  $\mu$ l) of all the drug solutions were infused into the lateral cerebral ventricle with the help of a slow injector apparatus (INCO, Ambala) and a 50  $\mu$ l Hamilton syringe, which delivered the solution at the rate of 2  $\mu$ l/min. Rectal temperature was measured at every 5 to 10 min intervals by Aplab 6 channel Telethermometer.

Post-mortem examination of each rat was done for confirmation of the placement of cannula in the lateral ventricle according to the procedures described previously (21).

## RESULTS

### *Effect of morphine on rectal temperature :*

Table I shows that cerebroventricular administration of 200  $\mu\text{g}$  morphine produced a consistent rise of rectal temperature between 1°C and 3.35°C in rats chronically exposed to higher seasonal air temperature (29.5° - 30.5°C) or to thermoneutral zone (27.5° - 28.5°C). This rise in rectal temperature was associated with salivation in rats when the body temperature exceeded 40°C.

### *Modification of hyperthermic effect of morphine :*

#### (i) *Pretreatment of rats with anticholinergic drugs :*

In this series of experiments which were carried out at high ambient temperature range of 29.0°C to 31.0°C, atropine (muscarinic receptor blocker), pentolinium and D-tubocurarine (nicotinic receptor blockers), and hemicholinium-3 (HC-3, an ACh synthesis inhibitor) were administered icv before morphine infusion. The results are shown in Table I and Fig. 1.

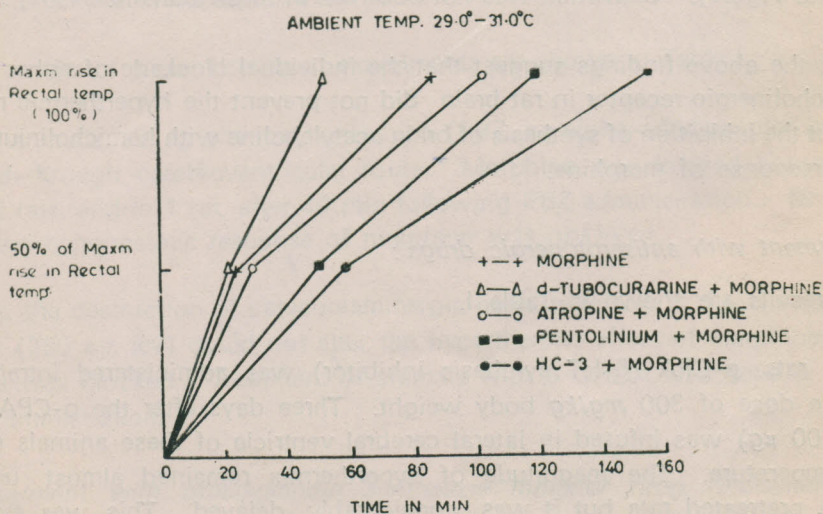


Fig. 1 : Time periods of hyperthermic responses of morphine after pretreatment with anticholinergic drugs. All the drugs were administered through icv route. (For details see text).

Prior administration of 50  $\mu\text{g}$  atropine in 4 rats did not influence the magnitude and response pattern of hyperthermic effect of morphine. The maximum rise, and rate

of rise in rectal temperature evident from the time taken for 50% of maximum rise (29.5 min), was almost similar to that observed with morphine alone.

Pre-treatment of 5 animals with pentolinium in varying doses (25  $\mu\text{g}$  in 1 rat, 50  $\mu\text{g}$  in 2 rats and 100  $\mu\text{g}$  in another 2 rats) could not attenuate the magnitude of hyperthermia though it delayed this response to some extent as evident from the Fig. 1. Whereas the time taken for reaching to maximum hyperthermia of 2.7° to 3.6°C was shortened (mean 52.75 min) following prior administration of 4-6  $\mu\text{g}$  D-tubocurarine in 4 rats; but this shortening of time period was mainly observed to be associated with the development of hyperexcitability and hypermotility in animals during the later phase of hyperthermic response and it was not due to the faster rate of initial rise in temperature as evident from the finding that the time period for 50% of maximum rise was not much different from that of control studies.

Prior administration of hemicholinium-3 (HC-3), 45  $\mu\text{g}$  in 2 rats and 90  $\mu\text{g}$  in another 2 rats, slowed the development of hyperthermic effect of morphine but did not alter the magnitude of hyperthermia. This slowing of hyperthermic response is evident from the observation that the time taken for maximum rise in temperature as well as that for 50% of maximum rise increased to double the value as compared to control experiments (see Table and Figure). Salivation was not observed in these animals.

Thus, the above findings suggest that the individual blockade of either muscarinic or nicotinic cholinergic receptor in rat brain did not prevent the hyperthermic response of morphine, but the inhibition of synthesis of brain acetylcholine with hemicholinium markedly altered this response of morphine.

(ii) *Pretreatment with antiserotonergic drugs:*

The results are shown in Table I.

In 2 rats, p-CPA (5-HT synthesis inhibitor) was administered intraperitoneally with a single dose of 300  $\text{mg}/\text{kg}$  body weight. Three days after the p-CPA treatment, morphine (200  $\mu\text{g}$ ) was infused in lateral cerebral ventricle of these animals exposed at 28°C air temperature. The magnitude of hyperthermia remained almost unaltered in these p-CPA pretreated rats but it was considerably delayed. This was evident from the findings that the time taken for maximum rise and that for 50% of maximum rise were greatly increased. This delay in response in animals was due to the loss of their righting reflex which developed 15-20 min after morphine administration, and it lasted for about 80-120 min. It was interesting to note that during this period of loss of righting reflex

the rectal temperature did not rise. It started to rise only when the animals regained righting reflex.

Another 2 rats were treated with intraventricular p-CPA, 100  $\mu\text{g}$  for three consecutive days in one animal, and 100  $\mu\text{g}$  for two successive days in another animal. Morphine was infused one day after the last treatment of p-CPA. No significant change in the pattern and magnitude of hyperthermic effect of morphine was observed and the animals did not show any abnormal behaviour. These two experiments were carried out at 30.0° and 34.5°C ambient temperatures respectively.

Further, the destruction of 5-HT nerve terminals by 5,6-DHT could not even modify the hyperthermic response of morphine in 2 experiments carried out at 27.0° to 28.0°C. The animals were pretreated with 5,6-DHT (150  $\mu\text{g}$ , icv) three days before morphine administration.

(iii) *Pretreatment with anticatecholaminergic drugs :*

These studies were carried out at low seasonal air temperature (17.5° to 21.5°C). It has already been reported earlier (21) that the hyperthermic effect of morphine in rats was always preceded with hypothermia at low ambient temperature.

The results of present series of experiments are shown in Table I.

In 3 animals, 20  $\mu\text{g}$  phenoxybenzamine (PBZ, an alpha-adrenoceptor blocker) was administered through cerebroventricular route. Morphine was infused between 80 and 90 min in 2 rats, and in 1 rat, after 40 min following PBZ administration. No significant change in the hyperthermic response of morphine was observed.

Further, the destruction of catecholaminergic nerve terminals in 5 rats with two doses of 6-OHDA (250  $\mu\text{g}$ , icv) could not alter the hyperthermic effect of morphine though the hypothermia was blocked. Treatment of animals with 6-OHDA was done 5 days before morphine administration.

(iv) *Pretreatment with prostaglandin synthetase inhibitor drug (indomethacin) :*

Since prostaglandins of E series are well known as endogenous mediator for infectious fever in almost all the species, their role in morphine induced hyperthermia was also investigated. The experiments were done at 30.0° to 32.5°C air temperature.

TABLE I : Effect of pretreatment of different drugs on morphine-induced hyperthermia in rats. Morphine was administered in a fixed dose of 200  $\mu$ g (icv) in all the experiments.  
(Values are mean  $\pm$  SD. Range is given in parenthesis)

No. of expts.	Seasonal air temp. range (°C)	Drug, dose and route	Time interval between drug and morphine	Rectal temperature			
				Latency of rise (min)	Maximum rise (°C)	Time period for rise (min)	
5	27.5-28.5	Morphine 200 $\mu$ g, icv	—	9.6 $\pm$ 6.8 (0-16)	2.74 $\pm$ 0.44 (2.3-3.35)	82.0 $\pm$ 37.0 (40-130)	29.0 $\pm$ 13.8 (16-50)
4	29.5-30.5	-do-	—	0	2.0 $\pm$ 0.76 (1.0-2.6)	87.5 $\pm$ 21.0 (65-110)	23.0 $\pm$ 11.6 (7-35)
4	29.0-31.0	Atropine 50 $\mu$ g, icv	25 min (20-45)	27.0 $\pm$ 34.8 (0-73)	1.94 $\pm$ 0.55 (1.5-2.6)	102.5 $\pm$ 24.6 (75-130)	29.5 $\pm$ 13.8 (10-42)
5	29.5-30.5	Pentolinium 25-100 $\mu$ g, icv	35 min (24-50)	34.0 $\pm$ 26.8 (15-80)	2.54 $\pm$ 0.28 (2.2-2.9)	119.0 $\pm$ 16.7 (100-140)	49.4 $\pm$ 8.4 (40-60)
4	29.0-29.5	Tubocurarine 4-6 $\mu$ g, icv	60 min (50-65)	0	3.03 $\pm$ 0.41 (2.7-3.6)	52.7 $\pm$ 12.9 (35-66)	22.0 $\pm$ 3.5 (18-25)
4	30.5	HC-3 45-90 $\mu$ g, icv	42 min (36-50)	58.0 $\pm$ 9.8 (50-68)	2.04 $\pm$ 0.32 (1.6-2.3)	*155.0 $\pm$ 57.4 (120-140)	*58.7 $\pm$ 27.8 (40-100)
2	28.0	p-CPA 300 mg/kg, ip	3 days	0	1.8 $\pm$ 1.95	205 $\pm$ 200	175 $\pm$ 95
1	30.0	100 $\mu$ g $\times$ 3, icv	1 day	1.2	1.0	60	50
1	34.5	100 $\mu$ g $\times$ 2, icv	1 day	0	2.5	35	24
2	27.0 $\pm$ 28.0	5,6-DHT 150 $\mu$ g, icv	3 days	12 $\pm$ 0	2.7 $\pm$ 3.0	160 $\pm$ 132	65 $\pm$ 45
3	19.0-20.5	PBZ 20 $\mu$ g, icv	70 min (40-90)	**	2.07 (1.65-2.7)	121 (100-150)	47 (32-72)
5	17.5-21.5	6-OHDA 250 $\mu$ g $\times$ 2, icv	5 days	27.4 $\pm$ 7.82 (15-35)	3.31 $\pm$ 0.67 (2.2-4.0)	132.0 $\pm$ 58.0 (45-190)	47.6 $\pm$ 29.4 (15-90)
3	30.0-32.5	Indomethacin 10 mg/kg, ip	20 min (20-25)	5 (0-14)	2.4 (2.25-2.5)	66 (60-73)	27 (20-36)

\*P < 0.05 as compared by Mann-Whitney U test to the results obtained with control experiments (morphine administered alone) at 29.5-30.5°C ambient temperature.

\*\* Observed hypothermia is not included in this Table.

In 3 rats, indomethacin (10 mg/kg, ip) was administered 20 min before morphine infusion. The subsequent hyperthermic response of morphine was unchanged in these indomethacin pretreated animals (Table I).

In another 2 rats, morphine was administered first, in the lateral cerebral ventricle, and indomethacin (10 mg/kg, ip) was injected during the initial rising phase of body temperature induced by morphine. No change in the hyperthermic effect of morphine was observed even in these animals.

### DISCUSSION

In rats, the most extensively studied species, the hypothermic action of morphine apparently results from its direct action on the thermoregulatory centre present within the PO/AH region (9,10). On the other hand, the hyperthermic effect of morphine can be produced from several sites of CNS including PO/AH region (4,8,11), midbrain raphe (19) and even spinal cord (18).

Gunne (7) reported that the hyperthermic effect of morphine in rats is not due to inhibition of heat loss system, but is the result of stimulation of heat production by increasing muscle tone and conserving the heat through vasoconstriction of cutaneous blood vessel of the tail. The inhibition of heat loss system is not responsible for hyperthermic effect of morphine, has also been evident from the present investigation. Thus, the initiation of salivation, a channel for heat loss in rats (8), was always observed when the rise in rectal temperature reached to a certain magnitude after morphine administration.

In accordance with the present concept of neurochemical theory of thermoregulation, Oka *et al.* (15) suggested that both the central and peripheral cholinergic mechanisms contribute to manifest the morphine-induced hyperthermia although they laid more emphasis on the peripheral mechanism because all the drugs were administered through systemic route in their investigation. Oka and Negishi (16) further confirmed the involvement of cholinergic system in morphine-induced hyperthermia in a specific strain of morphine non-tolerant rats, and they excluded the role of serotonergic or catecholaminergic system in this hyperthermic effect of morphine. It is also evident from the present study that pretreatment of animals with p-CPA (5-HT synthesis inhibitor), phenoxybenzamine (alpha-adrenoceptor blocker), 6-hydroxydopamine or 5,6-dihydroxytryptamine (nerve terminal degenerator of catecholamine and serotonergic pathways respectively) could not block or attenuate the hyperthermia elicited with central administration of morphine. Surprisingly, in our study the cerebroventricular administration of cholinergic nicotinic

or muscarinic receptor blocker drugs did not modify the hyperthermic effect of morphine whereas the Japanese workers (15,16) have shown that systemic injection of these receptor blockers have considerably attenuated morphine-induced hyperthermia. The difference in observation between these two studies might be due to either (i) the drugs administered through cerebroventricular route could not penetrate deeply in the central cholinergic receptor sites responsible for morphine-induced hyperthermia or (ii) both the muscarinic and nicotinic receptors were taking part at the central level to induce this hyperthermia, therefore blocking one receptor type at a time had not altered the effect of morphine on the other receptor, which subsequently led to hyperthermia. On the other hand, when the brain acetylcholine level was decreased by pretreatment of rats with hemicholinium, the hyperthermic response of morphine was profoundly delayed. This clearly indicates that the central cholinergic mechanism has a definite role in morphine-induced hyperthermia, though the post-synaptic receptor sites through which morphine acts, remain unclear.

Since catecholamines and serotonin are mostly located in nerve terminals within the PO/AH region(6) and their alteration of metabolism did not influence the hyperthermic effect of morphine, it is likely that morphine might not act on the controlling system at PO/AH region for producing its hyperthermic action, rather it may act on the effector system suggested to be located at posterior hypothalamus or at extrahypothalamic sites which are dominant with cholinergic neurones (20). Electrophysiological studies by Bradley and his colleagues (1) have reported that almost all the morphine sensitive neurones of brain stem effector system of rats are also sensitive to acetylcholine. Thus, it appears that morphine-induced hyperthermia is mediated via a central cholinergic system in rats. Further, Myers and Yaksh (14) have suggested that acetylcholine is not likely the transmitter substance mediating the effector mechanism involved in heat production in rats. It is interesting to note in our study, that the hyperthermic response of morphine after pretreatment of rats with hemicholinium was not associated with salivation in animals, suggesting that such attenuation was not the result of activation of heat loss system, rather it supports the idea that morphine brings its hyperthermic effect through the activation of central heat production system in which cholinergic links are present and hemicholinium suppresses this morphine-induced heat production through inhibition of cholinergic neurones.

Further, the study suggest that the hyperthermia produced by morphine in rats is not due to the stimulation of prostaglandin synthetase enzyme with subsequent release of prostaglandin of E series, as the prostaglandin synthetase inhibitor, indomethacin, did not prevent it. This confirms also the results obtained by Milton (12) in cats.



## REFERENCES

1. Bradley, P.B., G.J. Bramwell and A. Dray. Studies of the effect of morphine and related substances on single neurones in the brainstem. In *"Drugs and Central Synaptic Transmission"*, eds. Bradley, P.B. and B.N. Dhawan, London. The Macmillan Press Ltd., p. 309-315, 1976.
2. Burks, T.F. and G.C. Rosenfeld. Central neurotransmitter participation in temperature responses to morphine in the rat. In *"Drugs, Biogenic Amines and Body Temperature"*, eds. Cooper, K.E., P. Lomax and E. Schonbaum. Basel, S. Karger, p. 204-206, 1977.
3. Clouet, D.H. Ed. *Narcotic Drugs : Biochemical Pharmacology*. New York, Plenum Press, 1971.
4. Cox, B., M. Ary, W. Chesarek and P. Lomax. Morphine hyperthermia in the rat : an action on the central thermostat. *Eur. J. Pharmacol.*, **36** : 33-39, 1976.
5. Dey, P.K., W. Feldberg, K. P. Gupta and Sabine Wendlandt. Further studies on the role of prostaglandin in fever. *J. Physiol. (Lond.)*, **241** : 629-646, 1974.
6. Fuxe, F. Evidence for the existence of monoamine-containing neurons in the central nervous system. VI. The distribution of monoamine terminals in the central nervous system. *Acta Physiol. Scand.*, Suppl. 247, **64** : 37-85, 1965.
7. Gunne, L.M. The temperature response in rats during acute and chronic morphine administration : a study of morphine tolerance. *Arch. Int. Pharmacodyn.*, **129** : 416-428, 1960.
8. Lomax, P. Drug and body temperature. *Int. Rev. Neurobiol.*, **12** : 1-43, 1970.
9. Lotti, V.J. Body temperature responses to morphine. In *'The Pharmacology of Thermoregulation'*, eds. Schonbaum, E. and P. Lomax. Basel, S. Karger, p. 382-384, 1973.
10. Lotti, V.J., P. Lomax and R. George. Temperature responses in the rat following intracerebral microinjection of morphine. *J. Pharmacol. Exp. Ther.*; **150** : 135-139, 1965.
11. Martin, G.E. and J.E. Morrison. Hyperthermia evoked by the intracerebral injection of morphine sulphate in the rat : the effect of restraint. *Brain Res.*, **145** : 127-140, 1978.
12. Milton, A.S. Morphine hyperthermia, prostaglandin synthetase inhibitors and naloxone. *J. Physiol. (Lond.)*, **251** : 27-28P, 1975.
13. Milton, A.S. and Sabine Wendlandt. Effects on body temperature of prostaglandin of the A, E and F series on injection into the third ventricle of unanaesthetized cats and rabbits. *J. Physiol. (Lond.)*, **218** : 325-336, 1971.
14. Myers, R.D. and T.L. Yaksh. Feeding and temperature responses in the unrestrained rat after injections of cholinergic and aminergic substances into the cerebral ventricles. *Physiol. Behav.*, **3**: 917-928, 1968.
15. Oka, T., M. Nozaki and E. Hosoya. Effect of p-chlorophenylalanine and cholinergic antagonists on body temperature changes induced by the administration of morphine to nontolerant and morphine-tolerant rats. *J. Pharmacol. Exp. Ther.*, **180** : 136-143, 1972.
16. Oka, T., and K. Negishi. Effect of neurohumoral modulators on the morphine-induced hyperthermia in non-tolerant rats. *Eur. J. Pharmacol.*, **42** : 225-229, 1977.
17. Paolino, R.M. and B.K. Bernard. Environmental temperature effects on the thermoregulatory responses to systemic and hypothalamic administration of morphine. *Life Sci.*, **7**: 857-863, 1968.
18. Rudy, T.A. and T.L. Yaksh. Hyperthermic effects of morphine : set point manipulation by a direct spinal action. *Br. J. Pharmacol.*, **61** : 91-96, 1977.
19. Samanin, R., S. Kon and S. Garattini. Abolition of the morphine effect on body temperature in midbrain raphe-lesioned rats. *J. Pharm., Pharmacol.* **24** : 374-377, 1972.
20. Shute, S.C. and P.R. Lewis. Cholinergic and monoaminergic pathways in the hypothalamus. *Br. Med. Bull.*, **22** : 221-226, 1966.
21. Uday Prakash and P.K. Dey. Studies on rectal temperature of rats in relation to seasonal air temperature and morphine administration. *Ind. J. Physiol. Pharmac.*, **24** : 205-215, 1980.