STUDIES ON RECTAL TEMPERATURE OF RATS IN RELATION TO SEASONAL AIR TEMPERATURE AND MORPHINE ADMINISTRATION

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Summary: The present findings demonstrate that seasonal air temperature does not only influence the basal core temperature of rats, but also modifies the physiological/pharmacological actions of drugs. Thus, at low ambient temperature, intracerebroventricular or intraperitoneal administration of morphine produces mainly hypothermia followed by a secondary rise in rectal temperature. On the other hand, at high ambient temperature, the drug produces hyperthermia only. The hypothermic response at low ambient temperature is abolished by pretreatment of rats with 6-hydroxydopamine but not with phenoxybenzamine administration. This suggests that catecholamine pathway in the central nervous system is involved in morphine induced hypothermic response. Further, the role of cholinergic neurons in such response is also indicated.

Key words: body temperature, hypothermia, morphine, intracerebroventricular infusion

INTRODUCTION

The role of biogenic amines like noradrenaline (NA), serotonin (5-HT) and acetylcholine (Ach) in the central mechanism of regulation of body temperature has been well recognised following their demonstration in the hypothalamus, their profound ability to influence body temperature following intracerebroventricular (i.c.v.) or microinjection into the hypothalamic region, and the evidence for their release in response to change in ambient temperature and excellent reviews on these works have appeared (6, 7, 8, 20, 21). Based on this biochemical basis of central thermoregulation, the alteration of body temperature produced by several centrally acting drugs has been explained through perturbation of metabolism of these amines (6, 13, 14, 24). But such drug-induced alteration of body temperature has been shown to be different when the experiments were carried out at different controlled ambient temperature at which the animals were exposed for a definite period (3, 11, 23, 26). But such short term studies at experimentally controlled ambient temperature are not akin to the exposure of the animals and consequent adaptation occurring during natural seasonal changes in ambient temperature. In order to throw some light on the participation of biogenic amines like NA and Ach in the interrelationship between different seasonal air temperatures (at which the animals were reared) and drug-induced alteration of body temperature in animals, the effect of centrally administered morphine was carried out in rats with the following advantages: that the site of action of morphine is mainly at the preoptic/anterior hypothalamic (PO/AH) region (13, 15, 16) which is the anatomical substrate for controlling system of heat production and heat loss mechanism, as well as this hypothalamic region is normally rich in biogenic amines (10). Secondly,
the principal action of morphine is hypothermia at low ambient temperature in rats (8), therefore the modification of this response following pharmacological manipulation of the metabolism of biogenic amines can be evaluated.

MATERIALS AND METHODS

The experiments were carried out on different groups of inbred male albino rats (Wistar strain, 150–250 g) reared up at ambient temperatures existing at different seasons of the year. Drugs (morphine hydrochloride, U.P. Govt. Opium Factory; 6-hydroxydopamine hydrobromide, Sigma Chemical Co., U.S.A.; phenoxybenzamine hydrochloride and hexocholinium-3 bromide, obtained from the Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University) were prepared in sterile 0.9% (W/V) normal saline, and ascorbic acid (1 mg/ml) was added in the solution of 6-hydroxydopamine for prevention of oxidation of the compound. All the drugs were administered through intracerebroventricular route (i.c.v.). In addition, morphine was also injected through intraperitoneal route (i.p.) in some experiments.

Administration of drug through intraventricular route:

Under nembutal anaesthesia (35 mg/kg i.p.) the animal was placed in the rat stereotoxic apparatus (INCO, Ambala) and a stainless steel guide cannula (21 gauge) having a shaft length of 4 mm was chronically implanted into the anterior horn of the right lateral ventricle (9). Acrylic dental cement was used to secure the cannula with a cheese-head screw driven into the skull.

After 7 days of postoperative period, the drug solutions were infused into the lumen of the lateral ventricle of the animal. For that, a 26 gauge stainless steel infusion needle connected to a 50 μl Hamilton syringe by thin polyethylene tubing, was passed through the guide cannula so that its tip lied a little (0.5 mm) beyond the guide cannula. A constant volume of 20 μl of all the drugs solutions were infused into the lateral cerebral ventricle by a slow injector apparatus (INCO, Ambala), which delivered the solutions at the rate of 2 μl/min.

Measurement of rectal temperature:

The animal was kept in a rectangular perspex box (18 x 5 x 6 cm) having several round holes on its wall for free ventilation of air.

Rectal temperature was recorded from Aplab 6 channel Telethermometer through a thermistor probe (Yellow Spring Co., U.S.A.). The probe was inserted 6 cm deep inside the rectum and was held in place by wrapping adhesive leucoplast around the base of the tail of the animal. Temperature was noted at every 5–10 min intervals.

Drug solutions were administered once the basal rectal temperature of rats was stabilized, which generally occurred within 30 min.
ambient temperature in rats (23), pharmacological manipulation of
duplication at different seasons of the year. 6-hydroxydopamine
hydrochloride and hemi-leucoplast around the base of inbred male albino rats (CF at 6-hydroxydopamine
delivered through intracerebro-ventricular, was passed through
intraocular tubing, was passed through
beyond the guide cannula. A
delivered the solution at
(18 x 5 x 6 cm) having several
channel Telethermometer through
probe was inserted 6 cm deep.
rectal temperature of rats were

Placement of cannula in lateral cerebral ventricle and distribution of drug solutions
in the brain tissue were confirmed at the end of the experiment. Bromophenol blue dye
(0.8%) in a volume of 20 μl was infused into the lateral cerebral ventricle of rat adopting
exactly the similar method as for infusion of other drug solutions. The extent of blue staining
on the wall of cerebral ventricles and brain tissue was ascertained by naked eye observation. The results obtained from those rats which failed to show such blue stainings of the
cerebral ventricles have not been included in the present investigations.

RESULTS

Rectal temperature at different ambient temperature:
The results are shown in Table I. It appears that the basal rectal temperature of
rats shifts to a new low level when the air temperature falls from the thermoneutral zone.

<table>
<thead>
<tr>
<th>No. of obs.</th>
<th>Seasonal air temp. range</th>
<th>Rectal temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>15.5° — 21.5°C</td>
<td>*36.99°±0.95°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33.75°—37.8°C)</td>
</tr>
<tr>
<td>12</td>
<td>23.0° — 24.0°C</td>
<td>*36.75°±0.50°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(36.0°—37.5°C)</td>
</tr>
<tr>
<td>10</td>
<td>27.6° — 28.5°C</td>
<td>37.92°±0.43°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(37.25°—38.5°C)</td>
</tr>
<tr>
<td>42</td>
<td>29.0° — 32.5°C</td>
<td>37.96°±0.33°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(37.35°—38.65°C)</td>
</tr>
</tbody>
</table>

* <0.001 as compared to rectal temperature at thermoneutral ambient temperature (27.5° — 28.5°C)

Thus, the mean rectal temperature in 10 rats was 37.92°C (37.25° to 38.5°C) at thermoneutral zone (27.5° to 28.5°C). But when they were maintained at an air temperature of 23° to 24°C, the mean temperature in 13 rats was stabilized at 36.75°C (36.0° to 37.5°C); and such decline in rectal temperature became more pronounced with the further fall in the air temperature (15.5° to 21.5°C).

On the other hand, at higher ambient temperature of 29.0° to 32.5°C, the mean rectal temperature (37.96°C) of 42 rats did not significantly differ as compared to that at thermoneutral zone.
Effect of morphine on rectal temperature:

(a) *Morphine administered through intra-cerebroventricular route (i.c.v.):*

In a pilot study, the different doses of morphine in 20 µl volume were administered to observe their effect on rectal temperature. 200 µg of morphine was found to produce a consistent effect on rectal temperature, and therefore, this dose was used throughout the investigation. The results are shown in Table II.

At an ambient temperature of 23°C to 24°C, morphine produced a biphasic effect on rectal temperature. Thus there was an initial hypothermia varying between 0.6°C to 1.1°C (mean 0.75°C) within 10 min, and the time period for attaining maximum hypothermia varied between 17 and 70 min (mean 39.25 min). This initial hypothermia was subsequently followed by hyperthermia. The rise in temperature varied between 1.3°C and 3.5°C (mean 3.2°C) and the time period for maximum rise varied between 120 and 170 min (mean 87.5 min).

This hypothermic effect of morphine became further accentuated with decrease in the ambient temperature. Thus, at an ambient temperature between 17.5°C to 19°C, morphine produced a maximum fall in rectal temperature varying between 1.3°C and 1.8°C (mean 2.07°C). The subsequent rise in rectal temperature in this group was found to vary between 1.8°C to 4.6°C (mean 2.54°C).

There were no significant differences observed regarding the latency of fall in rectal temperature, the initial rate of fall in temperature, and the initial rate of rise in temperature.

On the other hand, the hypothermic response of morphine was absent at thermoneutral ambient temperature or at warm ambient temperature; the only response being hyperthermia. The maximum rise in rectal temperature at thermoneutral zone (27°C to 28.5°C) was between 2.3°C to 3.35°C (mean 2.74°C) and this rise was attenuated to a small degree at warm ambient temperature (29.5°C to 30.5°C) as shown in Table II. The initial rate of rise in rectal temperature was not different in these two groups. The latency for rise in rectal temperature became apparent from time taken for 50% of maximum rise in rectal temperature.

The latency for rise in rectal temperature showed that the hyperthermic response commenced immediately after morphine infusion in several rats, and in other cases it showed a mean value of 9.6 min i.c.v. or i.p., administration of 0.9% sterile normal saline did not influence the normal rectal temperature.

(b) *Morphine administered through intraperitoneal route (i.p.):*

The results are shown in Table II. It was observed that morphine administered in rats exposed at low ambient temperature (18°C to 21°C) produced a pronounced
Table II: Effect of morphine on rectal temperature of rats at different seasonal air temperatures.

(Values are mean ± SD. Range is given in parenthesis)

<table>
<thead>
<tr>
<th>No. of expts.</th>
<th>Seasonal air temp. range (°C)</th>
<th>Morphine dose &amp; route</th>
<th>Latency of fall (min)</th>
<th>Maximum fall (°C)</th>
<th>Time period for max. fall (min)</th>
<th>Time period for 50% of max. fall (min)</th>
<th>Maximum rise (°C)</th>
<th>Time period for max. rise (min)</th>
<th>Time period for 50% of max. rise (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>17.5 - 21.5</td>
<td>200 μg, iv</td>
<td>4 ± 0.03 (0-10)</td>
<td>2.07 ± 0.67</td>
<td>69.3 ± 42.8</td>
<td>20.9 ± 16.3</td>
<td>2.54 ± 1.03</td>
<td>82.9 ± 32.5</td>
<td>34.3 ± 23.9</td>
</tr>
<tr>
<td>4</td>
<td>23.0 - 24.0</td>
<td>200 μg, iv</td>
<td>2.8 ± 0.05 (0-10)</td>
<td>0.75 ± 0.31</td>
<td>39.25 ± 22.2</td>
<td>21.5 ± 13.4</td>
<td>3.2 ± 0.31</td>
<td>87.5 ± 22.1</td>
<td>41.7 ± 11.1</td>
</tr>
<tr>
<td>4</td>
<td>18.0 - 21.0</td>
<td>40 mg/kg, ip</td>
<td>0</td>
<td>3.47 ± 1.74</td>
<td>92.5 ± 21.0</td>
<td>28.7 ± 12.5</td>
<td>0.66 ± 0.63</td>
<td>25.6 ± 33.1</td>
<td>8.7 ± 11.8</td>
</tr>
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</table>

Latency of rise (min)

<table>
<thead>
<tr>
<th></th>
<th>9.6 ± 6.8 (0-16)</th>
<th>2.74 ± 0.44 (2.3-3.35)</th>
<th>82 ± 37.01 (40-130)</th>
<th>29 ± 13.02 (16-60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2.0 ± 0.76 (1.0-2.6)</td>
<td>87.5 ± 21.01 (66-110)</td>
<td>23 ± 11.66 (7-35)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.77 ± 0.97 (1.4-2.15)</td>
<td>76.4 ± 44.3 (40-140)</td>
<td>25 ± 8.16 (15-35)</td>
</tr>
</tbody>
</table>

*P < 0.01 as compared to maximum fall in rectal temperature at 22.0°C - 24.0°C ambient temperature.
in temperature varying between 1° to 5°C (mean 3.4°C) which reached within 70 to
120 min (mean 92.5 min).

The commencement of fall in rectal temperature following morphine was fast
and to be almost immediate, and the initial slope of fall was similar to that observed with
morphine administration. But subsequent rise in rectal temperature was very much
smaller (0° to 1.1°C) as compared to that of i.c.v. administration of morphine.

At warm ambient temperature of 28.5° to 38.5°C, morphine produced a
hyperthermia of about 1.4° to 2.15°C (mean 1.77°C), which reached within 40 to 90
min (mean 75 min).

Thus, the general pattern of response following e.tner i.c.v. or i.p. injection of
morphine at cold ambient temperature were found to be similar except that the hypothermic
effect of morphine given i.p. at cold ambient temperature was very much less in comparison
to that of i.c.v. morphine.

It has been observed that salivation occurred when the rise in rectal tempera-
ture reached to a certain magnitude following administration of morphine either through
i.c.v. or i.p. route.

Modification of hypothermic effect of morphine:
(i) Pretreatment with 6-hydroxydopamine (6-OHDA):

The results are shown in Table III. In 5 rats remained exposed to low ambient
temperature of 17.5° to 22.0°C, 250 μg of 6-OHDA was administered through the cere-
broventricular route once daily for 2 days. At the end of 5 days from the last injec-
tion, 200 μg morphine (i.c.v.) was administered. The usual hypothermic response of
morphine was abolished in this 6-OHDA pretreated rats. But the hyperthermic effect was
influenced in such pretreated animals.

(ii) Pretreatment of rats with phenoxybenzamine (PBZ):

The results are shown in Table III. In 3 rats, 20 μg phenoxybenzamine was
administered through i.c.v. route. In 2 rats, morphine 200 μg (i.c.v.) was adminis-
tered between 80 to 90 min, and in 1 rat after 40 min, following PBZ administration. The
animals were maintained at low ambient temperature of 19.0° to 20.5°C. The hypoth-
emic and hyperthermic response of morphine in such PBZ-pretreated rats were not signifi-
cantly altered as is apparent from Table III.
TABLE III: Hypothermic effect of morphine after treatment with 6-hydroxydopamine (6-OHDA), phenoxybenzamine (PBZ) and hemicholinium-3 (HC-3).
(Values are mean ± SD. Range is given in parenthesis)

<table>
<thead>
<tr>
<th>No. of expts.</th>
<th>Seasonal air temp. range</th>
<th>Drugs, dose &amp; route</th>
<th>Time interval between drug &amp; morphine</th>
<th>Rectal temperature</th>
<th>Racial temperature</th>
<th>Racial temperature</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Latency of fall</td>
<td>Maximum fall</td>
<td>Time period for maxm. fall</td>
<td>Maximum</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(min)</td>
<td>(°C)</td>
<td>(min)</td>
<td>(°C)</td>
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<tr>
<td>5</td>
<td>17.5 - 21.5</td>
<td>6-OHDA, 250 μg, icv + Morphine 200 μg, icv</td>
<td>5 days</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
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<tr>
<td>2</td>
<td>19.0 - 24.0</td>
<td>6-OHDA, 250 μg, icv + Morphine 40mg/kg, ip</td>
<td>5 days</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
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<tr>
<td>3</td>
<td>19.0 - 20.5</td>
<td>FBZ, 20 μg, icv + Morphine 200 μg, icv</td>
<td>70 min (40 - 90 min)</td>
<td>3.33</td>
<td>1.45</td>
<td>24</td>
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<tr>
<td>2</td>
<td>23.0 - 24.0</td>
<td>HC-3, 60 μg, icv + Morphine 200 μg, icv</td>
<td>40 min</td>
<td>0</td>
<td>*6.0</td>
<td>125</td>
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*HC-3 alone produced 3.0°C fall in rectal temperature prior to morphine administration.
Pretreatment with hemicholinium-3 (HC-3):

The experiments were carried out at ambient temperature range of 23.0°C to 24.0°C. The results are shown in Table III. In 2 rats, when HC-3, 80 μg and 90 μg respectively were given i.c.v. 40 to 45 min prior to the administration of 200 μg, i.c.v. morphine the drug HC-3 itself produced fall of 3°C in rectal temperature of both rats, and following morphine infusion, there was further fall of 3°C in both rats. The tendency of return of temperature to normal was not observed in any case even after 5 hours following morphine infusion.

In one rat HC-3, 90 μg (i.c.v.) was infused alone and a fall of 4.5°C rectal temperature occurred. The temperature returned to normal after 4 hours of HC-3 infusion in this case.

DISCUSSION

The present investigation shows that at thermoneutral zone of seasonal ambient temperature (27.5°C to 28.5°C) the basal rectal temperature of rat is generally around 37.5°C; but it undergoes considerable diminution (35.98°C) pari passu with low season air temperature (15.5°C - 21.5°C). Generally, this variation of normal rectal temperature in relation to seasonal ambient temperature may not usually be revealed, when the rest of the animals and the experiments are carried out at a controlled ambient temperature which is generally set around 22°C to 25°C.

That the air temperature can become an important determinant in modifying the physiological functions or altering the pharmacological actions of a drug has been apparent from the present investigations. Thus, the cerebroventricular (i.c.v.) infusion of 200 μg morphine in rats maintained at low ambient temperature between 17.5°C to 21.5°C produces biphasic response (hypothermia followed by hyperthermia). But the same i.c.v. infusion of morphine produces only hypothermia when the rats were maintained at thermoneutral zone (27.5°C to 28.5°C) or at high ambient temperature between 29.5°C to 30.5°C. The similar changes on rectal temperature were also obtained with intraperitoneal (i.p.) administration of morphine at low and high air temperature. These results corroborated well with the earlier findings of Pado and Bernard (23) following morphine injection into anterior hypothalamus of rat. The dependence of drug induced alteration in rectal temperature on the ambient temperature has also been reported by other workers (11,18,26). Thus, the peripheral thermal drive is an important determinant in influencing the body temperature responses to the drug.

It thus appears that the conflicting results often reported in literature on the regulatory responses to i.c.v., i.p. or intrahypothalamic injections of drugs may have been due to the experiments carried out at different ambient temperatures as already pointed out by Blixt as suggested.
Morphine Hypothesia in Rats

It has been well established that the hypothermic action of morphine administered through central or systemic routes is mediated mainly from the PO/AH region of hypothalamus (13, 15, 16), and Lotti et al. (17) have shown that this morphine induced hypothermia in rats mainly results from reduction of metabolic heat production, and not through activation of heat loss mechanism.

That this hypothermic effect of morphine is very much dependent on the noradrenergic pathways has been apparent from the present investigation. The morphine induced hypothermia observed at low ambient temperature was abolished following i.c.v. infusion of 6-hydroxydopamine (6-OHDA), a drug which almost specifically causes degeneration of the axonal fibres and nerve terminals of catecholamine neurons (12) without damaging the postsynaptic neuronal noradrenergic receptors. In other words, it follows from this observation that morphine most probably brings its hypothermic action by acting on these noradrenergic nerve terminals and not on the noradrenergic receptors of postsynaptic neurons.

An idea about the type of NA receptor located in the catecholamine pathway was provided from the present observation that phenoxybenzamine (PBZ), an alpha-adrenergic receptor blocker, did not inhibit or annul morphine induced hypothermia significantly, which indicated that postsynaptic NA receptor located in the heat production pathways did not belong to alpha-adrenergic type. From these observations, it may be speculated that in rats, at cold ambient temperature, the signals from the cold thermosensitive sensors of the skin and those from hypothalamic sensors normally activate the catecholamine pathway in PO/AH region and the release of NA from these pathways stimulate the neurons operating the heat production control system in order to maintain the body temperature in cold environment. This possibility gains support from the observation of hypothermia following injection of small amount of NA through i.c.v. route (9), into anterior hypothalamus (2) or counteraction of pilocarpine induced hypothermia with injection of NA at PO/AH region (13) in rats. In the light of the above facts, the fall in rectal temperature following morphine administration (i.c.v. or i.p.) at low ambient temperature, can be explained through the inhibition or prevention of release of NA from noradrenergic nerve terminals resulting in withdrawal of the "tonic drive" of these NA neurons from those responsible for heat production system. The evidence that morphine can inhibit the release of NA catecholamine nerve terminals was recently shown by Montel et al. (19). Bruinvels and Sourkes (4) and Bruinvels and Kemper (5) also showed that harmaline and tetrahydronaphthylamine induced hypothermia in rats was prevented after combined inhibition of biosynthesis and depletion of NA.
On the other hand, the promotion of hypothermic effect of morphine at cold ambient temperature following pretreatment of rats with hemicholinium-3 (HC-3), an Ach synthetase blocking drug, supports the involvement of cholinergic neurons in the heat production pathways. This has been further strengthened from the observation that HC-3 produces a marked fall in rectal temperature of rats at low ambient temperature. The involvement of cholinergic neurons in the heat production system has also been implicated by other workers (20-22). Recently Yaksh and Yamamura (25) reported that morphine inhibits resting and evoked release of Ach from neurons.

All these observations taken together likely to indicate in rats that a noradrenergic-cholinergic link operates for control of heat production at PO/AH region of hypothalamus, particularly at cold air temperature, and morphine probably produces hypothermia through impairment of this mechanism.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. J. Nagchaudhuri for extending laboratory facilities in the department, and to University Grants Commission, New Delhi for partial financial assistance to this project.

REFERENCES

The effect of morphine at cold ambient m-3 (HC-3), an Ach synthesis inhibitors in the heat production observation that HC-3 itself ambient temperature. The system has also been implicated in rats that a noradrenergic-C/AH region of hypothalamus produces hypothermia through extending laboratory facilities by Delhi for partial financial

e In our view during the last decade.

Jul-Sept 1980