PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 156, 523-526 (1977)

Effects on Temperature of Morphine Injected into the Preoptic/Anterior Hypothalamus, Medulla Oblongata, and Peripherally in Unrestrained and Restrained Rats[1, 2] (39971)

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Intracerebral injections of small doses of certain drugs alter body temperature in the same direction as do larger systemic doses, suggesting that these agents influence body temperature primarily by acting on central temperature controls. For example, oxotremorine and morphine injected directly into the preoptic/anterior hypothalamic (PO/AH) region of restrained rats cause hypothermia similar to that obtained after larger peripheral injections (1, 2). Pyrogens placed in the PO/AH region also cause fever at dose levels much smaller than those effective peripherally (3).

While these previous studies implicate the primary temperature control in the PO/AH region in drug-induced effects on body temperature, much less is known about the influence of drugs on temperature controls in the medulla oblongata (MED region). The existence of medullary temperature controls is indicated by the demonstration (4) that heating and cooling the medulla oblongata evoke changes in body temperature and behavior which parallel the effects of thermal stimulation of the PO/AH region. The effects of medullary thermal stimulation are not mediated through the PO/AH region since they still occur after destruction of this latter region. There is evidence that prostaglandin E_1 and pentobarbital, which increase core temperature when injected into the PO/AH region, produce hypothermia after intramedullary injections (5, 6) and that the MED temperature control is insensitive to doses of bacterial endotoxins that cause fever after PO/AH administration (5, 7). The primary purpose of the present experiments was to extend our comparisons of responses and relative sensitivities of the PO/AH and MED temperature controls to drugs which are well known to alter body temperature. The first drug chosen was morphine.

Materials and methods. Single-injection cannulae (23 gauge), with obturators of corresponding length, were fitted into a threaded nylon pedestal and protected with a Teflon cap using techniques similar to those described earlier (8). Under pentobarbital sodium anesthesia, these cannulae were implanted into adult, male rats (300 g) of the Holtzman strain. Coordinates derived from the brain atlas of Pellegrino and Cushman (9) were used to place the cannula tips into the PO/AH region (stereotaxic coordinates: lateral 0.0 mm, vertical 7.4 mm below the brain surface) of 23 rats. In the anterior-posterior plane, the cannula sites fell into two clusters: an anterior group primarily in the PO region but overlapping into the anterior portion of the AH region (anterior 7.8–8.5 mm) and a smaller cluster in the posterior portion (anterior 6.6–7.0 mm). Cannulae were placed into the MED region of 11 other animals at sites (lateral 0.0 mm and vertical 7.4 mm below the brain surface, just posterior to the transverse sinus) chosen on the basis of prior experience (4). Dental acrylic was used to secure the pedestal to stainless-steel screws driven into the calvarium. Testing began 7 days after surgery.

Solutions of morphine sulfate (Merck, Sharpe and Dohme) were made with nonpyrogenic 0.9% saline (Abbott Laboratories). In pilot experiments we determined a range of doses which appeared to produce dose-related changes in body temperature

[1] Supported by the National Institute of Neurological and Communications Disorders and Stroke Grant NS-10046 and by the Leland Fikes Foundation.

[2] Presented at the meeting of the Federation of American Societies for Experimental Biology, April 1976, Anaheim, California.

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ISSN 0037-9727

when injected into the PO/AH region. In the studies reported below, we then injected these doses into the PO/AH and MED regions to determine the temperature change evoked and the relative sensitivities of the two regions. All doses of morphine refer to the salt. Contamination by pyrogens was avoided by sterilizing injection cannulae, glassware, and microliter syringes at 200° for 2 hr and by using only commercial nonpyrogenic disposable syringes for making solutions.

Rectal temperature (T_{re}) was measured by inserting a Yellow Springs International thermistor probe 6 cm into the rectum and holding it in place until a stable reading was obtained on an electronic thermometer (Digitec Corporation). T_{re} was recorded at 15-min intervals until a consistent reading was obtained. Then the cap and obturator were removed, and 1 μl of either saline or drug solution was injected over 30 sec using a manual microdrive. The injection cannula was left in place for an additional 30 sec before replacement of the obturator. T_{re} was recorded for 5.5 hr after the injection. A paired t test was used to make statistical comparisons between responses to morphine and saline. Comparable levels of significance were obtained using both maximum changes in T_{re} and thermal response indexes (10), a measure of the total change in temperature over time. Unless otherwise indicated, animals were unrestrained in individual wire cages between temperature measurements. When restraint was used, the animals were placed in plastic holders (EconoCage) especially designed for the species. Injections were spaced at least 48 hr apart.

The PO/AH region was destroyed by electrolysis or through a series of ethanol injections in selected animals with MED cannulae (7). For electrolytic lesions, direct current (2 mA, 10 sec) was passed between an anodal electrode in the brain and a steel cathode in the rectum. The animals receiving ethanol were given 5 μl at the rate of 1 μl/min on the first day. A volume of 10 μl was then infused similarly on each of the next 3 days. After PO/AH destruction, T_{re} was generally elevated about 0.5° compared to prelesion levels regardless of which method was used to make the lesions.

To determine the sites of injection and the extent of lesions, the rats were killed with an overdose of pentobarbital, and the brains were perfused with saline and Formalin. Serial sections 35 μm thick were cut from the frozen brain tissue and stained using a modification of the Kluver-Barrera technique (11). Positions of the cannula tips were reconstructed on copies of line drawings taken from the brain atlas of Pellegrino and Cushman (9).

Results. Microinjection of morphine into the PO/AH region caused hyperthermia, but the sensitivity of the anterior and posterior clusters appeared to differ. Figure 1 (left) indicates responses to morphine injected into the more anterior sites in "novice" animals which had not previously received the drug. Hyperthermic responses to 50-μg ($P < 0.001$) and 25-μg doses began within 15 min and generally did not return to preinjection levels within the 5.5-hr recording period. A dose of 12.5 μg did not cause hyperthermia at anterior sites, but at the sites near the posterior border of the AH region this dose elevated T_{re} 1.4–2.4° ($P > 0.05$; Fig. 1, right). It is unlikely that responses to injections of morphine at the anterior sites were due to diffusion to a more sensitive posterior region because the responses developed more rapidly after injection into the anterior region.

Novice rats exhibited insignificant responses to morphine injections at MED sites located within 2 mm of the midline from the level of the pontine-medullary junction to the level of the obex (Fig. 2). When any response at all occurred, it was hyperthermia after 25 μg and hypothermia after 50 μg. Since the changes in T_{re} after MED administration of morphine were not significant, MED injections were repeated

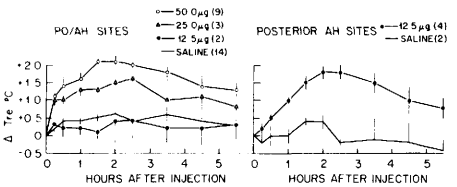

FIG. 1. Responses to injection of morphine into the anterior and posterior sites within the PO/AH region. In this and following graphs, results are given as means ± SE, and the number of animals tested at each dose is indicated in parentheses.

in animals in which the PO/AH region had been destroyed to determine if the low sensitivity was perhaps due to suppression of MED responses by the intact PO/AH region. The response to morphine was not enhanced by destruction of the PO/AH region (Fig. 2, lower right).

Because the hyperthermias recorded after PO/AH morphine in the present experiments contrasted with the decreases in T_{re} reported by Lotti *et al.* (1), who used restrained animals, we also gave morphine injections to restrained rats. Five of six rats developed hypothermia after morphine was injected into the PO/AH region (Fig. 3, left), an effect opposite to that produced by the same dose when animals were not restrained (see Fig. 1). In other experiments morphine was given ip to both restrained and unrestrained rats (Fig. 3, right). Hyperthermia ($P < 0.001$) developed in unrestrained rats, while the restrained rats became hypothermic ($P < 0.01$).

Discussion. Morphine caused hyperthermia when injected into the PO/AH region of unrestrained rats. Yet the same doses had no consistent effect when injected into the MED region, at sites within the region shown to be thermosensitive in previous experiments (4), even after destruction of the PO/AH region. These findings provide further evidence that the two control regions differ in their sensitivity to local drug administration. If the thermosensitive control in the MED is relatively insensitive to a drug, as in the case of morphine, changes in the MED temperature brought about by the drug acting on the PO/AH region might indirectly limit the change in body temperature. Thus the MED control would act as a defense against extreme deviations in body temperature (4). Data supporting the existence of such a control has been collated by Bligh (12) from experiments on changes in thresholds of thermoregulatory responses during anesthesia, on the upper fever limit,

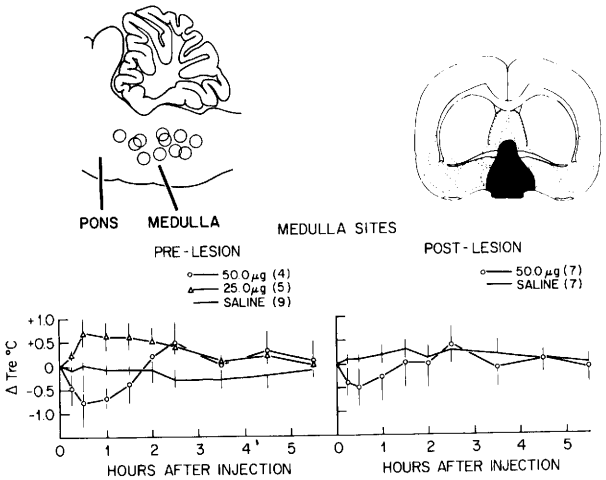

FIG. 2. Responses to morphine injected into MED sites. The insets above indicate injection sites in the medulla oblongata projected on a midsagittal section (all within 2 mm of midline) and the extent of a PO/AH lesion in a representative animal. PO/AH destruction did not appreciably alter the response to morphine.

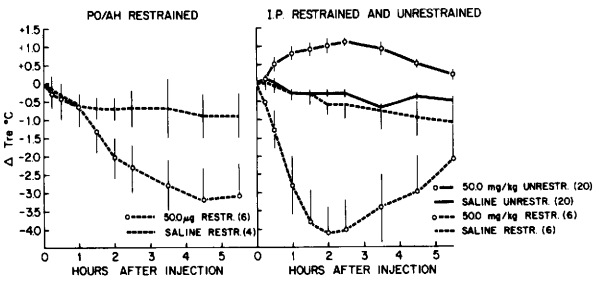

FIG. 3. Effect of restraint on responses to PO/AH and ip injections of morphine.

and on the core temperature required to cause maximal shivering.

Lotti *et al.* (1) concluded that morphine acts on PO/AH temperature controls from observations of hypothermia after both iv (to 50 mg/kg) and PO/AH injections (50 μg) in rats restrained in plastic cages. While our results lead to a similar conclusion with regard to the site of action, we found hyperthermia after central and peripheral administration of similar doses of morphine in unrestrained rats. Paolino and Bernard (13) have also reported hyperthermia in unrestrained rats given morphine peripherally. However, when we administered morphine to restrained rats, T_{re} decreased. Restraint has been shown to cause hypothermia even when no drugs were given (14–17), and restricting movement in morphine-dependent rhesus macaques (17) caused precipitous falls in body temperature much greater than the effects of restraint in untreated animals. The nature of the interaction between restraint and morphine is not clear but, since restraint reversed the effect of morphine action on the PO/AH, it may be that restraint can influence the primary temperature control directly.

These experiments provide additional support for the concept that the primary temperature control in the PO/AH region is more sensitive to drugs than is the secondary control in the MED region. In the case of the PO/AH control, there was some evidence for quantitative differences in response to morphine at different sites. An additional finding was that restraint can reverse the response to both peripheral and central morphine.

Summary. Changes in T_{re} were determined in unrestrained rats after injections of morphine into the PO/AH and MED regions. Injections into the former site consistently caused hyperthermia, while medullary injections caused only inconsistent small responses. Destruction of the PO/AH region did not enhance the response to medullary injections of morphine. Restraint reversed responses to PO/AH and ip administration of morphine, resulting in hypothermia. These results provide additional evidence for the concept of a sensitive primary temperature control in the PO/AH region and a secondary control, which responds minimally to drugs, in the medulla.

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Received April 25, 1977. P.S.E.B.M. 1977, Vol. 156.