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Morphine hyperthermia in the rat: its attenuation by physostigmine

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Summary

1. In rats, a subcutaneous injection of morphine (2·5 mg/kg) produced hyperthermia which was greatly attenuated by an intraperitoneal injection of physostigmine (0·1 mg/kg), but not of neostigmine (0·08 mg/kg) and promptly reversed by a subcutaneous injection of nalorphine.

2. It is concluded that the hyperthermia is a specific response to morphine, central in origin, and the result of diminished acetylcholine (ACh) release from central cholinergic neurones.

Introduction

In the experiments to be described, the effects of physostigmine, neostigmine and nalorphine on the hyperthermia produced in rats by moderate doses of morphine injected subcutaneously were investigated.

In rats, subcutaneous injections of morphine produce either a fall or a rise in body temperature according to dosage. Hypothermia appears to be the response to larger, hyperthermia to smaller, doses (Herrmann, 1942 ; Winter & Flataker, 1953 ; Gunne, 1960 ; Chodera, 1963). The hypothermia is certainly due to a central action of morphine since it occurs also when morphine is applied by microinjection into the anterior hypothalamus (Lotti, Lomax & George, 1965). The hyperthermia probably also results from a central action of morphine although this has not been proved, but indirect evidence for this view is obtained by the present experiments.

Schaumann suggested in 1957 that central actions of morphine may be due to inhibition of acetylcholine (ACh) release from central cholinergic neurones. Since then, it has been shown that morphine reduces the acetylcholine release from cortical and subcortical structures of the brain (Beleslin & Polak, 1965 ; Sharkawi & Schulman, 1969 ; Jahamandas, Pinsky & Phillis, 1970). There are also indications that a cholinergic link is involved in the central regulation of body temperature in the rat since it was found in this species that blockage of central cholinergic transmission led to a rise in body temperature (Kirkpatrick & Lomax, 1967, 1970).

If the morphine-induced hyperthermia in the rat is the result of diminished acetylcholine release from central cholinergic neurones involved in hypothalamic thermoregulation, an intraperitoneal injection of physostigmine might attenuate the hyperthermia since the brain cholinesterase becomes inhibited. On the other hand, an intraperitoneal injection of neostigmine should not have this effect, because this inhibitor of cholinesterase does not pass the blood-brain barrier and therefore acts peripherally only (Goldstein, Aronow & Kalman, 1969). From the effects obtained

with the two cholinesterase inhibitors, it would thus be possible to find out if the hyperthermia results from a central action of morphine, and if a cholinergic link is involved in this action. From the effects obtained with nalorphine, on the other hand, it should be possible to conclude if the hyperthermia is a specific morphine effect because, in that case, it should be abolished by this specific morphine antagonist.

Methods

Female Sprague-Dawley rats weighing 170–200 g were used. The experiments were carried out at room temperature varying between 23 and 25° C. During the experiments, the rats had free access to water and food and were allowed to run freely in their cages. Rectal temperature was taken as an index of body temperature and measured by a thermistor mounted in a rectal probe connected to a telethermometer (Yellow Spring Instrument Company, Yellow Springs, Ohio). The thermistor probe was inserted 4·5 cm deep into the rectum. Two control measurements were taken for each rat at an interval of 30 min before the injection of the drugs. The average value of the two measurements was taken as the initial temperature and subtracted from the rectal temperature determined after the drug injection, which was done every 30 min during a period of 150 minutes.

In a few experiments, the cholinesterase activity of the brains was determined. Three groups of four rats were used for this purpose. The rats were killed by decapitation, the brains immediately removed and the cholinesterase activity was measured manometrically as described by DuBois & Mangun (1947).

Drugs used. Morphine sulphate, nalorphine hydrochloride, physostigmine sulphate, neostigmine bromide. All doses given in the text refer to the base. The drugs were injected in a volume of 1 ml/kg dissolved in 0·9% NaCl solution.

Results

The hyperthermic effect of a subcutaneous injection of 2·5 mg/kg morphine is shown by the interrupted curve with the full circles in Fig. 1. The hyperthermia reached its maximum after 60 min and temperature returned to nearly the pre-injection level after 150 minutes. The morphine-induced hyperthermia was greatly attenuated by an intraperitoneal injection of 0·1 mg/kg physostigmine given immediately after the morphine injection, as shown by the curve with the open triangles, whereas the curve with the open squares shows that the physostigmine alone did not affect temperature. The attenuation produced by the physostigmine of the morphine-induced hyperthermia, is statistically significant (Student's *t* test) at 60, 90 and 120 min intervals ($P<0·01$) but not at 30 and 150 min intervals. An equimolar dose of neostigmine (0·08 mg/kg) injected intraperitoneally immediately after the morphine injection did not attenuate the morphine-induced hyperthermia. This is shown by the curve with the open circles.

The injection of physostigmine in the dose that attenuated the morphine-induced hyperthermia caused some inhibition of brain cholinesterase, whereas the injection of neostigmine did not have this effect. This was evident when the brain cholinesterase was determined in rats killed 30 min after an intraperitoneal injection of

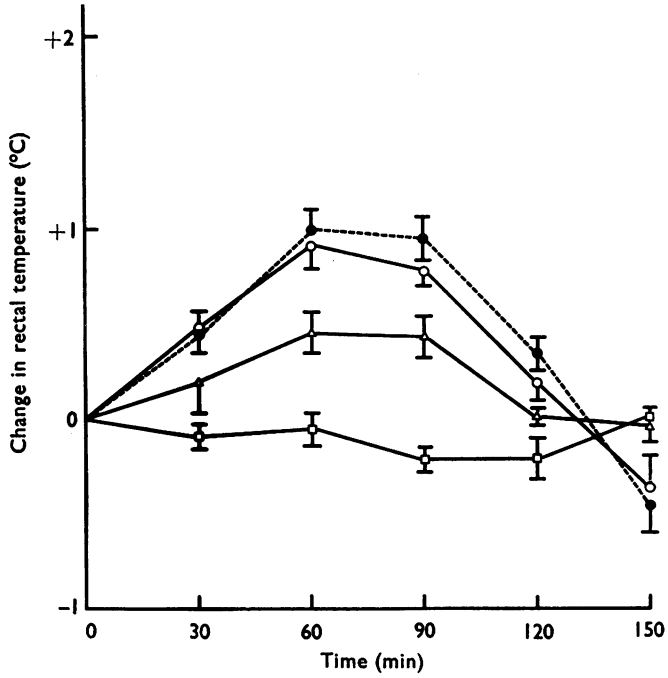

FIG. 1. Changes in temperature of rats after the following injections given at zero time: (- - -●- - -) 2·5 mg/kg morphine, subcutaneously; (—○—) 2·5 mg/kg morphine, subcutaneously and immediately afterwards 0·08 mg/kg neostigmine, intraperitoneally; (—△—) 2·5 mg/kg morphine, subcutaneously and immediately afterwards 0·1 mg/kg physostigmine, intraperitoneally; (—□—) 0·1 mg/kg physostigmine, intraperitoneally. Each point represents the mean change of six–eight rats ± standard error, and each curve was obtained from a different group of rats.

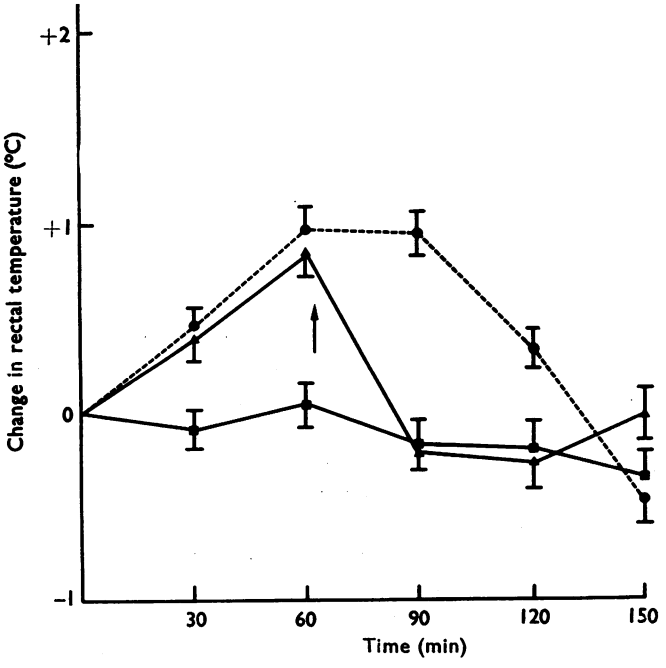

FIG. 2. Changes in temperature of rats after the following injections given at zero time: (- - -●- - -) 2·5 mg/kg morphine, subcutaneously (same curve as in Fig. 1); (—▲—) 2·5 mg/kg morphine, subcutaneously and 60 min later, at the arrow, 1·25 mg/kg nalorphine, subcutaneously; (—■—) 1·25 mg/kg nalorphine, subcutaneously. Each point represents the mean change of six–eight rats ± standard error, and each curve was obtained from a different group of rats.

0·1 mg/kg physostigmine or of 0·08 mg/kg neostigmine. In these animals, the brain cholinesterase activity was 90% and 98% respectively of the value obtained for the brains of rats killed 30 min after an intraperitoneal injection of 1 ml/kg 0·9% NaCl solution.

The temperature effects obtained with nalorphine are illustrated in Fig. 2. Injected subcutaneously in a dose of 1·25 mg/kg nalorphine by itself did not affect rectal temperature (curve with full squares) but it promptly reversed the morphine-induced hyperthermia (curve with full triangles). For comparison, the effect of morphine alone is shown in Fig. 2 by the interrupted curve with the full circles which is the same curve as that given in Fig. 1.

Discussion

The prompt reversal of the morphine-induced hyperthermia in the rat by nalorphine, which by itself did not influence body temperature, recalls the similar antagonistic effect which nalorphine has on the morphine hyperthermia produced in the cat when these drugs are injected into the cerebral ventricles (Banerjee, Feldberg & Lotti, 1968) and suggests that in the rat too, the hyperthermia is a specific response to morphine. This suggestion is supported by the findings that the magnitude of the morphine hyperthermia in the rat is dose dependent and that the morphine congener levorphanol produces a hyperthermia in rats which is also promptly reversed by its antagonist levallorphan (Ferron & Sharkawi, unpublished experiments).

The suggestion of a cholinergic link being involved in the central regulation of body temperature in the rat is based on the findings that, in this species, hypothermia occurs on iontophoretic application of acetylcholine into the region of the rostral hypothalamus, and hyperthermia on microinjections of atropine into this region. The hyperthermia was explained by blockage of central cholinoceptive sites to the neurotransmitter acetylcholine (Kirkpatrick & Lomax, 1967, 1970). On the assumption that the central actions of morphine, including the hyperthermia, result from its ability to diminish acetylcholine release from central cholinergic neurones, the attenuation of the morphine hyperthermia produced by intraperitoneal injections of physostigmine is readily explained by inhibition of cholinesterase activity of the brain which was produced by these injections. The results thus support the idea of a cholinergic link being involved in the temperature regulation of the rat. On the other hand, the finding that neostigmine did not attenuate the morphine hyperthermia is readily explained by the inability of the cholinesterase inhibitor to pass the blood brain barrier, which was evident also from the fact that the cholinesterase activity of the brain did not become reduced. Finally, the difference in the effect of the two cholinesterase inhibitors on the morphine hyperthermia provides indirect evidence that the hyperthermia results from a central action of morphine.

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