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The Apparent Hyperalgesic Effect of a Serotonin Antagonist in the Tail Flick Test is Mainly Due to Increased Tail Skin Temperature

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TJØLSEN, A., A. LUND, P. K. EIDE, O.-G. BERGE AND K. HOLE. *The apparent hyperalgesic effect of a serotonin antagonist in the tail flick test is mainly due to increased tail skin temperature.* PHARMACOL BIOCHEM BEHAV **32**(3) 601–605, 1989.—It has been suggested that reduced activity in raphe-spinal serotonergic systems induces hyperalgesia. In rats, the serotonin antagonist metergoline (0.5 mg/kg intraperitoneally) reduced tail flick latency by 0.92 sec ($p<0.001$) and increased tail skin temperature by 2.4°C ($p<0.001$) when measured 50 min after injection. Multiple regression analysis with tail flick latency as dependent variable and tail skin temperature and metergoline/vehicle as independent variables revealed a highly significant effect of tail temperature on tail flick latency. The increase of tail skin temperature explained a reduction of tail flick latency of 0.64 of the 0.92 sec observed [$B = -0.267 \pm 0.034$, $t(37) = -7.75$, $p<0.0001$]. When the effect on tail skin temperature was taken into account, metergoline reduced tail flick latency by 0.28 sec [$B = -0.284 \pm 0.114$, $t(37) = -2.50$, $p<0.05$]. Metergoline (0.5 and 2.0 mg/kg) did not significantly alter plantar paw skin temperature or the response temperature in the increasing temperature hot plate test. Thus, the observed effect of metergoline on tail flick latency is primarily due to an effect on tail skin temperature. The possibility exists that the remaining effect of metergoline may be due to inadequate correction for the skin temperature change, and it is concluded that the study provide no clear evidence for a tonic inhibition of nociception by serotonergic systems.

Serotonin Metergoline Nociception Pain Tail flick test Skin temperature Skin blood flow Rats

SKIN temperature might be of critical importance for the results of nociceptive tests involving thermal stimuli, both in man (5, 7, 15, 16) and in animals (10, 11, 19, 25, 26). With regard to the tail flick test it has been postulated that the tissue temperature at which the response occurs may be more consistent than the response latency (26). In line with this, we have previously found a strong negative correlation between tail flick latency and pretest tail skin temperature in rats and mice (4, 12, 28), demonstrating that fluctuations in tail skin temperature under normal laboratory conditions may seriously influence the tail flick latency.

Serotonergic systems play an important role in body temperature regulation and associated vasomotor control (8, 14, 18, 22–24, 27). Serotonin is furthermore assumed to be important in the regulation of nociception (3, 21, 30). With growing evidence that skin temperature influences the results in important tests for nociception, it has become necessary to reexamine the role of 5-HT in nociception.

We have reported that selective lesioning of descending serotonergic pathways with intrathecal administration of 5,6-dihydroxytryptamine in rats leads to a decrease in tail flick latency which can be entirely explained by a concomitant increase in tail skin temperature (28). Previous studies have shown that administration of the serotonin receptor antagonist metergoline may lead to decreased latencies in the tail flick and conventional hot plate test (1,2). In this study, we have reexamined the effect of metergoline in the tail flick test taking into account possible effects on tail skin temperature. The effect of the antagonist was also evaluated in the increasing temperature hot plate test, a nociceptive test which does not depend on response latencies and which has been found to be sensitive to a broader range of analgesics than either the conventional hot plate test or the tail flick test (17).

METHOD

Animals

Male Sprague-Dawley rats (Mol:SPRD, Møllegaard, Denmark) were used. Forty rats (280–340 g) were used to establish a dose-response curve for metergoline in the tail flick test, forty rats (245–310 g) in the tail flick/temperature experiment and forty rats (258–310 g) in the increasing temperature hot plate test. The animals were housed at 21–24°C, 3–4 to a cage with free access to

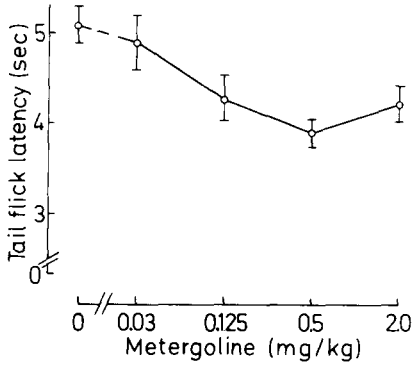

FIG. 1. The effect of metergoline in the tail flick test (mean ± SEM, n = 7–8 in each group). Metergoline was given IP 50 min before testing.

food and water. They were kept on a 12/12 hr light/dark cycle with lights on at 7.00 a.m.

Drugs

Metergoline (Farmitalia) was dissolved in 0.2 M methanesulphonic acid, and the solution was diluted with 0.9% NaCl to a final concentration of methanesulphonic acid of 0.01 M. The injections were made intraperitoneally in a volume of 5 ml/kg. An equal volume of vehicle (0.01 M methanesulphonic acid in 0.9% NaCl) was used as control.

Testing Procedures

Nociceptive testing was performed following a single-blind design, the observer being unaware of the drug treatment in each experiment.

For tail flick testing, the animals were habituated to the test situation daily during the week before testing. The habituation consisted of progressively longer exposures to the testing apparatus (5–20 sec), including actual tail flick testing on the last two days. The animals were brought to the test room 22–26 hr before testing, which took place between 10.00 a.m. and 1.00 p.m., and the ambient temperature during testing was 22.0 ± 1.0°C. The animals were tested 50 min before and 50 min after the injection of metergoline or vehicle.

In order to establish a dose-response relationship for metergoline, tail flick latencies were recorded after injection of metergoline (0.030, 0.125, 0.50 and 2.0 mg/kg) or vehicle as described below without measuring the skin temperature (Fig. 1). The lowest dose with maximum effect, 0.5 mg/kg, was used in the subsequent tail flick/temperature experiment.

Recording of tail flick latency and tail skin temperature was performed as described in detail elsewhere (29). Tail flick latency was measured by focusing a beam from a light bulb on the tail, the centre of the heated area being 13 mm from the tip of the tail. The time from onset of stimulation to withdrawal of the tail was recorded. Beam intensity was adjusted to give tail flick latencies of approximately 4 sec. The tail skin temperature was measured on the dorsal surface, 10 mm proximal to the centre of the heated area, by means of a copper-constantan thermocouple probe (0.05 mm copper-constantan wire, the junction measuring 0.1 × 0.6 mm). The junction of the probe was mounted freely suspended on a plastic arm, and rested lightly on the tail during testing. The beam was controlled from the keyboard of a computer, and tail flick latency and tail skin temperature were recorded by the computer.

For testing, the rat was lifted out of the cage, placed on the testing apparatus, and gently restrained by holding the proximal part of the tail. The plastic arm was lowered onto the tail. The temperature reading stabilized within approximately 1 sec. Within a few seconds the tail skin temperature was recorded and the beam was turned on. If struggling occurred during testing, the animal was returned to the home cage and no further attempt was made to test it again until the next session.

For the increasing temperature hot plate test the animals were handled for three consecutive days before testing, and were brought to the test room 2–4 hr before testing, which took place between 10.00 a.m. and 12.00 a.m. The animals were tested 50 min after injection of drug. The ambient temperature during testing was 22.0 ± 1.0°C.

Recording of response temperatures in the increasing temperature hot plate test was performed using a modification of a method previously described (17). A specially designed hot plate equipment was used, where an aluminium plate was heated and cooled by Peltier elements in contact with its lower surface. The Peltier elements heated or cooled the plate depending on the direction of the applied current. The temperature of the plate was controlled by an electronic feed-back circuit connected to a thermistor in contact with the plate. The plate measured 12 × 27 cm and was enclosed by an unlidded perspex box, 30 cm high. Starting temperature was 42.0°C, and the temperature was increased by 3.0°C/min. The temperature of the plate when the first hind paw lick occurred was recorded as the response temperature. It was decided to employ a cut-off value of 52.0°C, but all animals responded at lower temperatures.

Twenty-four of the rats from the hot plate experiment were used for measurement of paw temperatures. Paw skin temperatures were measured 45 min after injection of vehicle or metergoline (0.5 or 2.0 mg/kg). A thermocouple probe (0.20 mm copper-constantan wire, the junction measuring 0.7 × 1.0 mm) was pressed against the skin on the plantar surface of the hind paw, between the treading-pads, while the rat was manually restrained. The rats were well adapted to being manipulated, and were not struggling during measurement. The temperature reading stabilized within 3–4 sec.

Statistical Procedures

Multiple regression analysis was used to analyze the interactions between treatment, skin temperature and tail flick latencies. Analysis of variance (ANOVA) was used to investigate the effect of metergoline on paw temperature and in the increasing temperature hot plate test. Student's *t*-test was applied when comparisons were restricted to two means.

RESULTS

Tail Flick Test

A dose-dependent effect of metergoline on tail flick latency was observed (Fig. 1). The effect was statistically significant ($p<0.05$, *t*-test) from 0.125 mg/kg upwards.

In the tail flick/temperature experiment, the difference in mean tail flick latency between the metergoline (0.5 mg/kg) and vehicle treated groups was 0.92 sec (metergoline: 2.81 ± 0.05, vehicle: 3.73 ± 0.11 sec, means ± SEM, $p<0.001$, *t*-test), the preinjection latencies being nearly identical (4.10 ± 0.10 vs. 4.27 ± 0.10 sec). This metergoline effect is similar to earlier findings (1). Simultaneously, the metergoline group showed a mean tail skin temperature 2.4°C higher than the vehicle group (29.0 ± 0.15 vs. 26.6 ± 0.34°C, means ± SEM, $p<0.001$, *t*-test).

Before administration of drug a highly significant negative

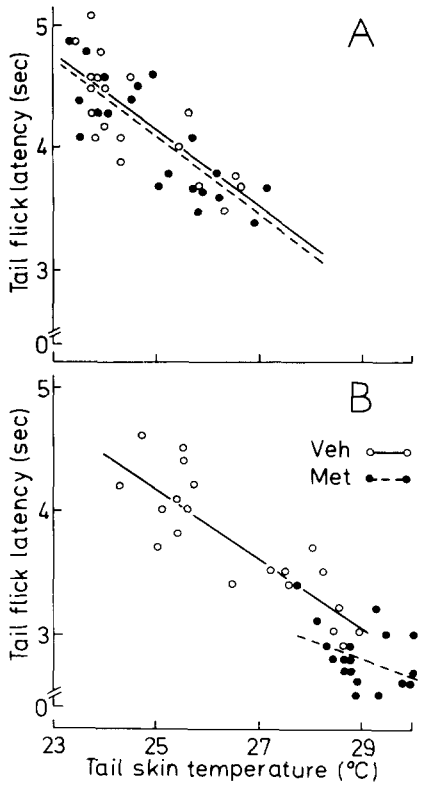

FIG. 2. Tail flick latency plotted against tail skin temperature. Data were obtained before (A) and 50 min after (B) injection of metergoline 0.5 mg/kg (Met) or vehicle (Veh) (n = 20 in each group).

correlation between tail flick latency and tail skin temperature was found in both groups (Fig. 2A, vehicle group: R = 0.78, intercept = 12.15, slope = −0.32, metergoline group: R = 0.82, intercept = 12.04, slope = −0.32).

After injection the vehicle group had decreased tail flick latencies and increased tail skin temperatures compared to the preinjection recordings. Multiple regression analysis showed that the increase of tail skin temperature could explain the decrease of tail flick latency, and there was no significant change of tail flick latency when skin temperature was taken into account. There was a negative correlation between tail flick latency and tail skin temperature similar to the results for the preinjection recordings (Fig. 2B, R = 0.86, intercept = 11.41, slope = −0.29).

The metergoline group showed, however, considerably lower tail flick latencies and higher skin temperatures after injection. These recordings showed considerably less variation in temperatures and latencies. Hence, the correlation coefficient is lower, and the regression data of less significance (R = 0.44, intercept = 7.32, slope = −0.16).

Multiple regression analysis of the data obtained 50 min after injection of metergoline or vehicle with tail flick latency as dependent and tail skin temperature and treatment as independent variables showed a high degree of overall multiple correlation (R = 0.92, $p<0.001$). Furthermore, it revealed a highly significant effect of tail skin temperature on tail flick latency [B = −0.267 ± 0.034 (SEM), $t(37) = -7.75$, $p<0.0001$]. The effect of treatment when skin temperature was taken into account was still significant [B = −0.284 ± 0.114 (SEM), $t(37) = -2.50$, $p<0.05$].

The increase of tail skin temperature obtained by metergoline thus explained a reduction of tail flick latency of 0.64 of the 0.92 sec observed. When the effect of change in tail temperature was taken into account, metergoline reduced tail flick latency by 0.28 sec.

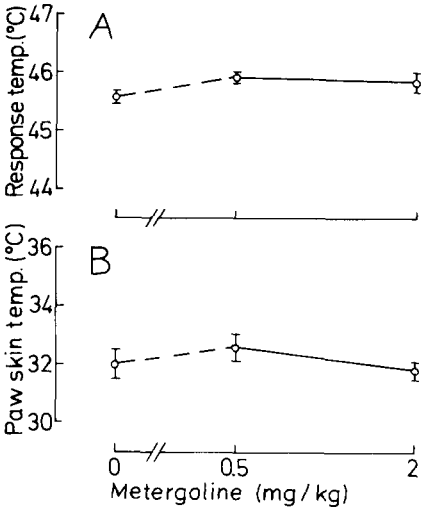

FIG. 3. (A) The effect of metergoline on response temperature (mean ± SEM) in the increasing temperature hot plate test (n = 13–14 in each group). (B) The effect of metergoline on paw skin temperature (mean ± SEM, n = 8 in each group).

Increasing Temperature Hot Plate and Paw Skin Temperature

Response temperatures after injection of vehicle, 0.5 and 2.0 mg/kg metergoline were 45.57 ± 0.13, 45.90 ± 0.12 and 45.82 ± 0.18°C, respectively (Fig. 3A). The effect of metergoline on response temperature was not significant, $F(2,37) = 1.259$, $p = 0.295$, ANOVA.

The effect of metergoline on paw skin temperature (Fig. 3B) was not significant, $F(2,21) = 0.993$, $p = 0.389$, ANOVA.

DISCUSSION

The results in this study confirm that systemic administration of the serotonin antagonist metergoline in rats is followed by a decrease in tail flick latency. Previously the interpretation has been that serotonergic systems exert a tonic inhibition on nociception. The findings in the present study show that most of the decrease in tail flick latency can be explained by a marked rise in tail skin temperature following administration of metergoline. The change in tail temperature explained all but 0.28 sec of a total decrease of 0.92 sec.

Furthermore, no hyperalgesic effect of metergoline could be demonstrated in the increasing hot plate test. A slight, but insignificant, elevation of response temperature was recorded. It has been shown that the increasing temperature hot plate test is sensitive to nonnarcotic analgesics (17), and seems to be well suited to detect minor changes in nociception. Previous studies have shown that selective neurotoxic lesions of the descending serotonergic pathways fail to alter the response temperature in the increasing temperature hot plate test (6).

A temperature threshold for pain independent of pretest skin and body temperature is found in man (9,20), and in the rat (26). In the tail flick test a strong heat stimulus is applied to the skin. Theoretical considerations make it reasonable to assume that the time it takes to heat the tissue surrounding the nociceptors to threshold is dependent on the pretest skin temperature. In the

increasing temperature hot plate test the heat stimulus is weaker and of much longer duration, probably allowing a temperature equilibrium between the paw skin and the hot plate to develop. One may therefore assume that pretest skin temperature plays a minor role in this test. Furthermore, no significant changes in paw skin temperature could be demonstrated in the present investigation. This may indicate that the regulation of blood flow in the tail and in the hind paws is different. However, the temperature of the plantar surface of the paw shows considerable regional variations, and the plantar skin is prone to get wet or contaminated with faecal material, which may alter the skin temperature. This makes recordings of skin temperature an inaccurate measure of paw blood flow. A change in paw blood flow after administration of metergoline, hence, cannot be excluded.

In all groups of rats that were investigated, a near linear negative relationship between tail skin temperature and tail flick latency was found. This is in agreement with several former studies (4, 12, 28, 29). When evaluating the tail flick latencies taking tail skin temperature into account, it is therefore possible to apply multiple regression analysis. The effect of drug on tail flick latency is then evaluated, removing the change in tail flick latency caused by the drug effect on tail skin temperature.

Although small, a certain effect of metergoline on tail flick latency remained when skin temperature was taken into account. Similar experiments have been done in mice, where no significant effect of the serotonin antagonists metergoline and metitepin on the tail flick latency could be demonstrated when the latencies were corrected for tail skin temperature (13). In these experiments, however, there was a somewhat weaker correlation between skin temperatures and tail flick latencies. Hence, the data were less suitable for multiple regression analysis, and the temperature effect was taken into consideration by applying a correction factor obtained from a large control material. When, as in the present study, the skin temperature is measured close to the heated area in rats, the correlation between skin temperatures and tail flick latencies is sufficiently strong to allow a multiple regression analysis. This strong correlation makes it possible to demonstrate a difference in tail flick latency too small to reach significance when using the tail flick test without correction for the skin temperature change.

However, in the increasing temperature hot plate test, no significant effect of metergoline on nociception could be demonstrated. The possibility exists that the observed effect in the tail flick test may be due to an inadequate correction for skin temperature. The temperature is recorded 10 mm from the centre of the heated area, and occasionally we have observed temperature gradients of up to 3°C/10 mm in this area of the tail (29). Another possibility is that there indeed exists a weak tonic descending inhibition of the spinally integrated, nociceptive tail flick reflex. However, the negative observation in the increasing hot plate test would then indicate that nociceptive responses which require more complex processing are modulated differently from the tail flick reflex.

There is strong evidence for an antinociceptive effect of serotoninergic agonists and serotonin reuptake inhibitors, hence suggesting an important role of serotonin in pain perception. However, the present data, taken together with other studies (6, 12, 13, 28), provide little evidence for the hypothesis of a tonic inhibition of nociception by serotonergic systems.

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