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Hyperalgesia Following Ischaemia of the Rat's Tail

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Summary

We have investigated the effects of ischaemia on responses to a subsequent noxious stimulus in rats. Tail flick latencies to a noxious thermal stimulus were determined by immersing the tail in water at temperatures ranging from 39 to 49°C. We then produced ischaemia by occluding the blood supply to the tail; ischaemia was terminated at the first signs of an escape response. Tail flick latencies were recorded immediately after termination of ischaemia and at 30 min intervals for another 2 h. Each rat acted as its own control. Tail flick latency decreased after ischaemia; we found a decrease of about 39% immediately after ischaemia, at immersion temperatures above 39°C. The duration of the hyperalgesia increased with increasing water temperatures. Thus noxious ischaemia of the rat tail induced hyperalgesia to subsequent noxious thermal stimuli. The hyperalgesia could have arisen through either central or peripheral mechanisms.

Introduction

Sensitization of nociceptors by cell metabolites released during trauma is thought to be one of the mechanisms causing hyperalgesia following injury [2,3]. Similar metabolites are released during tissue ischaemia, so apart from the pain induced during ischaemic episodes (myocardial infarct or intermittent claudication, for example), one would predict that such episodes would be followed by a period of local hyperalgesia. We have therefore investigated the sensitivity of the rat's tail to noxious stimuli following an ischaemic episode.

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We used a modified tail flick test to apply controlled noxious stimuli. To induce ischaemia we applied a tourniquet around the base of the tail. The tourniquet was left in place until the rat's behaviour indicated that the ischaemia was noxious. Some of the results have been reported briefly to the Physiological Society of Southern Africa [7].

Methods

Animals

Male Sprague–Dawley rats weighing 250–300 g were used in all experiments. Different groups of 10 rats were used for each test.

Tail flick test

For the modified tail flick test [5,12] the rats were placed in restrainers which allowed free movement of the tail. The tail was submerged in a water bath at a controlled temperature and the time taken from submergence to the first coordinated motor response, measured on a stopwatch, was taken as the tail flick latency. A mean of 3 successive measurements was taken. To avoid tissue damage, animals which failed to respond within 30 sec had their tails removed from the water.

Ischaemia

Ischaemia was induced by application of an inflatable cuff to the base of the restrained rat's tail. The cuff was connected to a sphygmomanometer which was inflated to a pressure of 200 mm Hg. The cuff was deflated as soon as the rats responded either by vigorous grooming, or by attempting to turn around or jump forward. A transient escape response within the first 2 min was ignored: in humans application of a sphygmomanometer cuff sometimes causes mild discomfort initially. The cuff was removed, however, if the rat persisted to show distress. The time taken from inflation of the cuff to the first signs of escape was measured. In 110 rats this time was 12.5 ± 0.2 min (mean $\pm$ S.E.). If the rat had not responded within 30 min the cuff was deflated, to avoid excessive tissue damage. Control experiments were done by leaving an uninflated cuff on the rat's tail for 12 min.

Experimental procedure

The rats were placed in the restrainers for 15 min prior to any testing. Tail flick latencies were measured prior to application of the cuff (basal values), immediately after deflation and then at regular intervals for 2 h (test values). Pilot experiments were conducted at an immersion temperature of 49°C, to establish approach time intervals between successive tests of the tail flick response, as well as the recovery period after the ischaemic episode. In the light of the results of the pilot experiments, tail flick latencies were determined at 30 min intervals following ischaemia, and immersion temperatures ranging from 26°C to 49°C were used. The order of tests at the different temperatures was randomized. In one group of rats the cuff was inflated for a set time period (5 min) and subsequent tail flick latencies tested at 49°C.

The experimental procedures were approved by the Animal Ethics Committee of the University of the Witwatersrand: in all cases stimuli were removed immediately the animals' behaviour indicated the stimuli were noxious. There were no lasting sequelae. We have complied with the proposals of the Committee for Research and Ethical Issues of IASP [18].

Results

Tail flick latency following repeated testing

Measurements were made of tail flick latency, in different groups of rats, following immersion of the tail in water at 49°C, with the test procedure repeated at different time intervals between 10 min and 60 min. No ischaemia was applied.

When the immersion test was repeated every 10 min, the tail flick latency decreased progressively. After 2 h the latency was about 1.3 sec (or about 30%) shorter than the initial latency, and the shortening was statistically significant. Thus repeated testing by water immersion at 10 min intervals induced significant hyperalgesia in its own right, as previously reported by Lynn [11]. A similar trend towards hyperalgesia was evident when the tests were repeated at 20 min intervals. However, when 30 min were allowed between tests (open circles, Fig. 1) there was no significant hyperalgesia.

Tail flick latency following ischaemia

Ischaemia induced an immediate hyperalgesia to a thermal stimulus. Tail flick

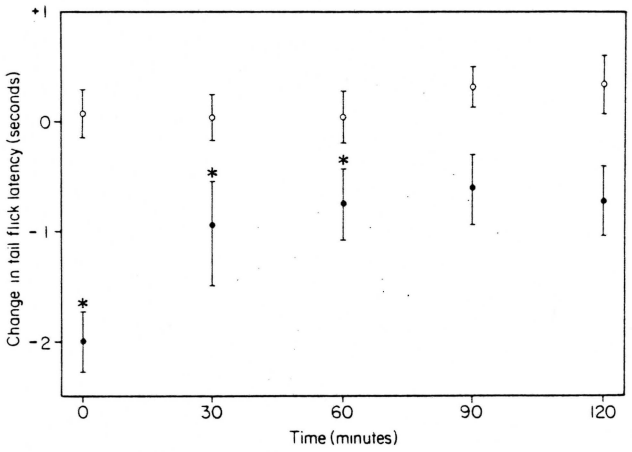

Fig. 1. Change in tail flick latency (mean ± S.E.) following repeated testing at 30 min intervals, by water immersion, with (●, n = 10) and without (○, n = 10) previous ischaemia. The change in latency varied significantly with time in the ischaemic group ($P = 0.013$ in one-way analysis of variance with Bonferroni correction for repeated measures) but not the control group. Points marked with an asterisk were significantly different from control. Initial tail flick latencies were 4.84 ± 0.41 sec for the ischaemic group and 3.56 ± 0.33 sec for the control group.

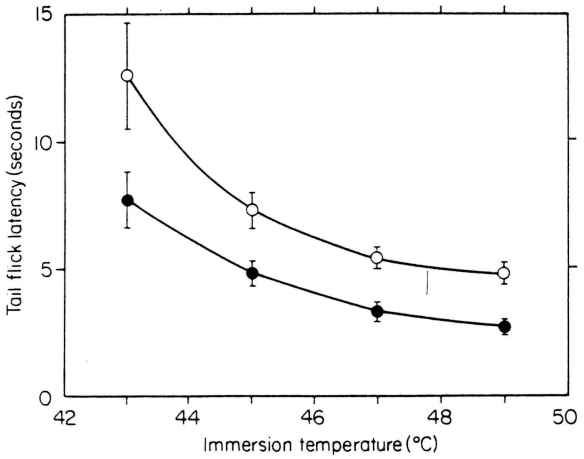

Fig. 2. Tail flick latency (mean ± S.E.) at different immersion temperatures immediately after removal of the ischaemic cuff (●, n = 10) and sham tourniquet (○, n = 10). There was a significant difference between the non-ischaemic group and the ischaemic group ($P < 0.01$, two-way analysis of variance), confirmed at each temperature by paired t tests.

latencies following water immersion at 49°C decreased by 1.80 ± 0.11 sec ($P < 0.01$, n = 40, paired t test), or about 39%, when the test was conducted immediately the rat responded to the noxious ischaemic stimulus.

There was a gradual recovery to the normal tail flick latency after ischaemia was terminated. The recovery was not evident when tail flick latency tests were repeated at 10 min intervals; the decrease in latency was statistically significant ($P < 0.05$, paired t test) at all time intervals tested, because repetitive testing itself induced hyperalgesia.

When tests were repeated every 20 min, recovery took place after 80 min. When the tests were repeated at 30 min intervals (Fig. 1), recovery was established by 90 min. Thus the rats appeared to be hyperalgesic for about 1.5 h following a single ischaemic episode, when tested by immersion at 49°C.

The degree and duration of the hyperalgesia depended on the temperature of the water in which the tail was immersed. Fig. 2 shows the decrease in tail flick latency immediately after termination of the ischaemia, at various immersion temperatures.

Tail flick latencies when tested at 0, 30, 60, 90 and 120 min after removal of the sham tourniquet (cuff applied for 12 min but not inflated), at tail immersion temperatures of 41–49°C, were not significantly different from basal tail flick values. Thus there was no hyperalgesia at any time or at any temperature following application of the sham tourniquet.

Fig. 3 shows the cumulative response curves following ischaemia for immersion temperatures between 41°C and 49°C. At temperatures below 41°C, the rats showed inconsistent responses to water immersion; many did not respond within 30 sec. At the higher temperatures the tail flick latency was significantly decreased following ischaemia at all temperatures. The hyperalgesia had disappeared within 30 min,

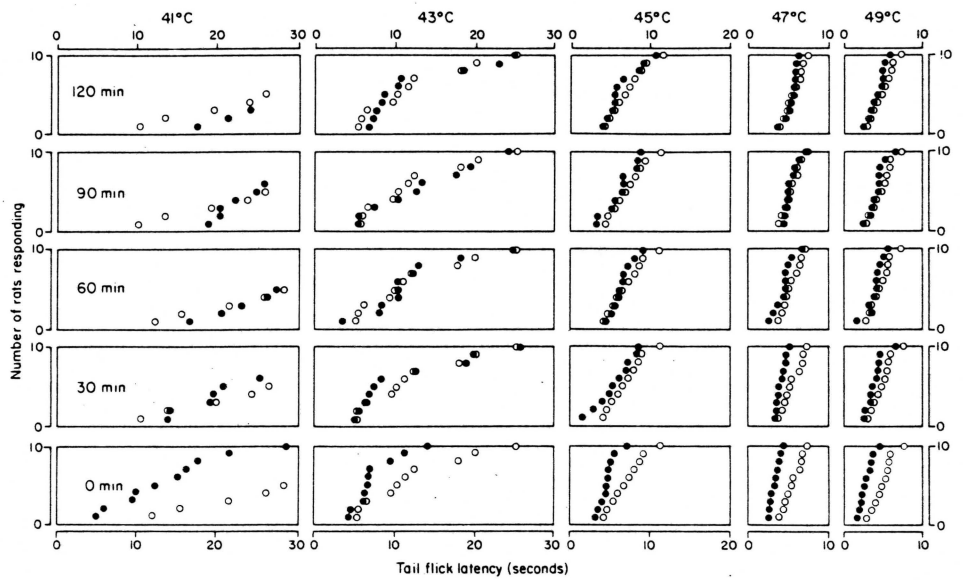

Fig. 3. Number of rats responding at various tail flick latencies when tested at 0, 30, 60, 90, and 120 min after termination of ischaemia at tail immersion temperatures of 41–49°C. Open circles represent tail flick latency recorded prior to ischaemia and the closed circles tail flick latencies after ischaemia. Differences in the cumulative curves were tested by chi-square tests, and the results are reported in the text.

when tested by immersion at 41°C and 43°C, by 60 min at 45°C and by 90 min at 47°C and 49°C. Thus the more noxious the test stimulus, the longer the hyperalgesia to that stimulus was evident following ischaemia.

Discussion

Ischaemia, produced by occluding the blood supply to the rat's tail, caused significant hyperalgesia to subsequent noxious thermal stimulation.

The time taken for individual rats to respond to application of the ischaemic cuff varied considerably. Therefore we could either standardize the time for which ischaemia was applied, or use the same physiological end point, namely that indicated by coordinated escape behaviour in each rat. We attempted to standardize the time of ischaemia at 5 min in one group of rats. This period produced no hyperalgesic response, and for ethical reasons we felt that we could not apply the cuff for a longer standard time period, since some rats would become unacceptably distressed. We decided to use the same end point even though there was a variability in the time taken for each rat to respond.

The tail flick test was used as a measure of the hyperalgesia subsequent to ischaemia. It is known that repeated thermal stimulation produces hyperalgesia [1,3,6,10], and we confirmed the presence of such hyperalgesia when thermal

immersions were repeated at 10 or 20 min intervals. To test for ischaemic hyperalgesia, we used 30 min intervals between tail immersion tests, at which no test-induced hyperalgesia was observed.

In both test and control groups a decrease in tail flick latency with increasing immersion temperature was observed. This result is consistent with the observations of Necker and Hellon [13], who found an increase in the firing rate of tail afferent nerve fibres with a stepwise increase in temperature. No hyperalgesia was observed after application of a sham tourniquet, but hyperalgesia was evident subsequent to ischaemia, at all immersion temperatures above 39°C. Immediately after ischaemia the magnitude of the hyperalgesia was not significantly different at the different temperatures above 39°C, but the duration of the hyperalgesia was dependent on the intensity of the subsequent noxious stimulus. The threshold temperature required to elicit a flexor response to tail immersion in normal rats has been reported as 43.7 ± 0.6°C [12]. After ischaemia we found that a threshold temperature as low as 41°C elicited a flexor response.

The decrease in the flexor response following ischaemia could have been caused by humoral mediators released during ischaemia, which were capable of sensitizing nociceptors. The concentration or effect of these mediators then could have decreased with time, following relief of the ischaemia. Possible mediators are hydrogen ions, kinins, prostaglandin, histamine, 5-HT and lactate [4,8,10,11,14,15]. Alternatively, the change in the flexor response could have been due to an increase in excitability of neurones of the spinal cord [16,17].

Our results indicate that ischaemia induces a significant hyperalgesia. Further experiments are necessary to determine the mechanism involved, which could be either peripheral or central, or both.

Acknowledgements

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