*Pharmacology Biochemistry & Behavior*, Vol. 40, pp. 197–201. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

# Hyperbaric Exposure and Morphine Alter the Pattern of Behavior in the Formalin Test

ODD-GEIR BERGE,[1] INMACULADA GARCIA-CABRERA AND KJETIL FURSET

*Department of Physiology, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway*

Received 20 March 1991

BERGE, O.-G., I. GARCIA-CABRERA AND K. FURSET. *Hyperbaric exposure and morphine alter the pattern of behavior in the formalin test.* PHARMACOL BIOCHEM BEHAV **40**(2) 197–201, 1991.—This study investigates the behavioral effects of morphine administration and exposure to high ambient pressure in the formalin test. Rats were simultaneously given formalin (0.1 ml, 5%) in a hind paw, and saline or morphine (2.5–10.0 mg/kg) subcutaneously. They were then exposed to ambient pressure of either 1 or 48 bar (compression rate: 3 bar/min; 1 bar is approximately equal to the pressure of 1 atmosphere) in a helium-oxygen atmosphere. The behavior of the animals was monitored for 35 min at stable pressure, starting 25 min after the injections. After morphine, the groups tested at 1 bar showed a dose-dependent reduction in pain-related activities such as licking, biting, clutching and protecting the injected paw but paw-elevation while resting was significantly increased after the highest dose. The 48-bar groups spent almost no time in these behavioral categories but showed an increase in apparently normal motor activity. Paw-jerking appeared to be a more robust response. The number of jerks was not altered by pressurization and was dose-dependently reduced by morphine at both pressures. The results show that hyperbaric exposure alters the response pattern in the formalin test, demonstrate the advantage of evaluating several behavioral criteria in this test and provide tentative evidence against pressure reversal of morphine analgesia.

Analgesia Formalin test Hyperbaric conditions Nociception Pressure reversal Rats Stress

THE formalin test has gained considerable recognition as a useful analgesimetric procedure. The test employs a continuous stimulus which generates behavioral (10) and electrophysiological (9) responses that persist from several minutes up to more than an hour. In this regard, the test differs distinctly from the more commonly used hot-plate and tail-flick tests which register short-lasting behavioral and reflexive responses to noxious stimuli of brief duration. The formalin test is sensitive to a wider range of analgesics than these tests (17, 19, 20) and has been considered to be a more adequate model for clinical pain (3).

The implementation of the formalin test varies with regard to site of injection, response criteria, data sampling and stimulus intensity (concentration and volume of formalin). Selection of response criteria may be particularly important for the validity of the test, but this issue has not been extensively studied in the rat. Usually, a single "pain" index is calculated for a defined time period, based on the accumulated time of one or several selected behavioral responses (3, 10, 12).

Another problem which has received little attention is the possible interference of the treatments evaluated with the ability of the subjects to perform the criterion responses. Motor impairment may, for instance, be incorrectly interpreted as alterations in nociception.

The present investigation was carried out in order to provide a better understanding of the behavioral responses of rats in the formalin test. A microcomputer was used for continuous scoring of all observed behavior, and the effects of a pharmacological treatment factor (morphine) and an environmental stress factor (compression and exposure to elevated ambient pressure) were investigated.

The stressor was chosen partly because it represents a standardized and highly reproducible procedure which has been shown to induce changes in motor activity in rats (13,14) and partly to obtain information which may contribute to the development of a test suitable for investigating pain and analgesia at high ambient pressure. Pressure interferes with the action of some drugs. The pressurization procedure used in this study antagonizes the behavioral effects of ethanol (13) and there is a well-documented pressure reversal of the effect of several anesthetics (16, 21, 22, 26). However, in spite of important practical and theoretical implications, little is known about possible interactions between pressure and commonly used analgesics.

## METHOD

### *Subjects*

Drug-naive male Sprague-Dawley rats (Mol:SPRD, Møllegård, Denmark) weighing 250–300 g at the beginning of the experiment were housed three to a cage. The light phase lasted from 0800 to 2000 hours and ambient temperature was maintained at 22–23°C. All experiments took place during the light period and animals belonging to the various treatment groups (n = 8 for each group) were tested in randomized order.

### *The Hyperbaric Chamber and the Compression Procedure*

Experiments were carried out in a steel hyperbaric chamber (approximately 24.5 l internal volume) fitted with an acrylic

[1]Requests for reprints should be addressed to Dr. Odd-Geir Berge, Astra Pain Control, S-151 85 Södertälje, Sweden.

window. Temperature was controlled by circulating water through a copper coil around the outside of the chamber. The gas temperature inside the chamber was registered by means of thermistor probes. Temperature and pressure data were continuously updated on a computer monitor and stored for later analysis. An electric fan ensured proper mixing of the gases inside the chamber and forced the gases through a cartridge containing soda lime to prevent $CO_2$ accumulation.

The animals were habituated to the chamber for 45 min immediately before injection of formalin. From 2 min after injection, the chamber was flushed with a mixture of 80% helium and 20% oxygen. Compression was started 4 min 20 s after injection at a rate of 3 bar/min so that the animals reached stable pressure 20 min after formalin injection. The period at pressure lasted 40 min. The 1-bar groups received the same treatment as the 48-bar groups except that compression was replaced by flushing with heliox. Oxygen partial pressure was maintained between 0.2 and 0.4 bar.

Analysis of the data recorded during compression and at 48 bar showed that the pressures were within 1% of the predefined values in all experiments.

The temperature of the chamber was adjusted to offset helium-induced hypothermia (7). The temperature settings were 30°C for the 1-bar groups and 34°C for the 48-bar groups. During the observation period, the recorded temperature deviated by less than 0.5°C from the set values.

*Handling and Testing Procedures*

All animals were gently handled for 1 min on two consecutive days and were tested on the third day.

In the chamber, each rat was free to move within an area of 210×110 mm. Two rats were tested at the same time but in individual enclosures. To facilitate observation, a mirror was positioned at a 45° angle at the back of each enclosure. The rats were allowed 45 min in the testing enclosure before injection of dilute formalin (100 µl, 5%) into the dorsal surface of the left hind paw. Morphine (morphine-HCl, local supplier; 2.5, 5.0 or 10.0 mg/kg dissolved in a volume of 5 ml/kg 0.9% NaCl) or an equivalent volume of vehicle was injected subcutaneously in the neck, immediately before formalin administration.

For 60 min starting at the time of injection, continuous video recordings were made through the window of the chamber. Subsequently, analysis of the behavior was performed by means of a computer program that allowed the observer to register categories of behavior in terms of accumulated time and number of events. The observer did not know the group assignment of the animals. Scoring started 25 min after injection of formalin and morphine (5 min after reaching stable pressure in the 48-bar groups) and scores were accumulated for 7 consecutive 5-min periods. All behavior was recorded as belonging to one of the following categories:

1) Focused, pain-related activity: Motor activity directed towards the injected paw, including licking, biting and shaking of the paw as well as clutching the paw tightly against the flank. 2) Modified, pain-related activity: Motor activity not directed towards the injected paw but modified as to protect it, e.g., limping during locomotion or partially elevating the injected paw during rearing or grooming. 3) Not pain-related motor activity: Motor activity not directed towards, or without protecting, the injected paw. 4) Resting with the injected paw elevated or clutched against the flank. 5) Resting without protecting the injected paw. 6) Paw-jerks involving the injected paw.

The accumulated time of each of the first five categories and the number of responses in category 6 were used to evaluate the behavior.

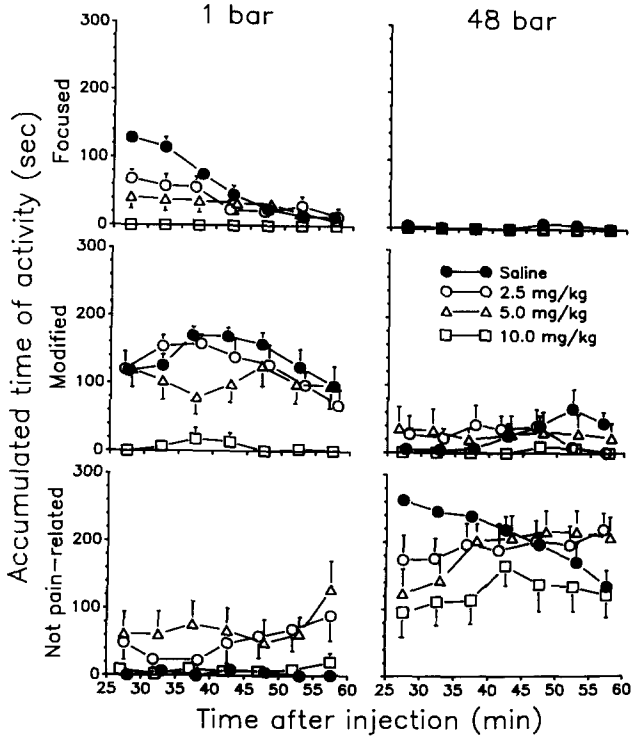


FIG. 1. Formalin test on rats at 1 or 48 bar ambient pressure: activity focused on the injected paw, modified as to protect it or apparently unrelated to the formalin injection. Data given as mean and S.E.M, n=8 in each group. Formalin and morphine or saline were injected at time 0. The time spent in each behavioral category was accumulated for 5 min at a time, starting at 25 min. Compression of the 48-bar groups started at 4 min 20 s and was completed at 20 min.

*Statistical Analysis*

The behavioral data were subjected to repeated measures analysis of variance (ANOVA) as detailed in the results. Significance was accepted at the 5% level.

## RESULTS

*Focused, Pain-Related Activity*

With the exception of paw-jerking, all active responses involving the injected paw or directed towards it were completely suppressed at 48 bar (Fig. 1, upper panel).

At 1 bar, the overall amount of activity as well as the dose-related reduction in activity after morphine was particularly pronounced during the first half of the session. Comparisons with the saline group (2 doses × 7 periods ANOVA) revealed significant group effects starting with the 5 mg/kg dose, $F(1,14) = 5.91$, $p<0.05$, and significant group × period interaction after all doses, including the 2.5 mg/kg dose, $F(6,84) = 5.39$, $p<0.0005$.

*Modified, Pain-Related Activity*

At 48 bar, the amount of activity modified as if to protect the injected paw was greatly reduced [Fig. 1, middle panel; $F(1,56) = 52.96$, $p<0.00001$ for pressure effect, 2 pressures × 4 doses × 7 periods ANOVA]. Furthermore, a significant interaction between pressure and dose, $F(3,56) = 5.89$, $p<0.002$, was found. At 1 bar, the 10 mg/kg group [$F(1,14) = 331.43$,

$p<0.00001$; 2 doses × 7 periods ANOVA], but none of the other groups had significantly lower scores than the saline group, while at 48 bar, no statistical difference between groups was present (no tendency to group effect, period effect or interaction, 4×7 ANOVA).

*Not Pain-Related Motor Activity*

The pressurized groups showed considerably more activity scored as unaffected by pain than the 1 bar groups [Fig. 1, lower panel; $F(1,56)=80.53$, $p<0.00001$ for pressure effect, 2 pressures × 4 doses × 7 periods ANOVA]. At 1 bar, only the groups that had received 2.5 and 5.0 mg/kg of morphine showed a measurable amount of such activity, with the level increasing towards the end of the session. There was only a tendency towards difference between groups, $F(3,28)=2.87$, $0.05<p<0.10$, and towards group × period interaction, $F(18,168)=1.60$, $0.05<p<0.10$, at 1 bar (4 doses × 7 periods ANOVA).

The groups at 48 bar displayed a considerable amount of apparently normal activity. Initially, morphine tended to reduce the level of activity in a dose-related manner, but the group difference was not statistically significant [$F(3,28)=2.70$, $0.10>p>0.05$, 4 doses × 7 periods ANOVA]. While the saline-treated animals gradually became less active with time, the opposite tendency was evident after morphine, and comparisons with the saline group demonstrated significant group × period interactions after all doses [2.5 mg/kg: $F(6,84)=5.37$, $p<0.0005$; 5 mg/kg: $F(6,84)=5.02$, $p<0.0005$; 10 mg/kg: $F(6,84)=4.29$, $p<0.002$; 2 doses × 7 periods ANOVA for each comparison].

*Time at Rest With the Injected Paw Elevated*

This category was all but absent at pressure (Fig. 2, upper panel). At 1 bar, there was only a nonsignificant tendency to overall difference between the groups [$F(3,28)=2.79$, $0.05<p<0.10$, 4 doses × 7 periods ANOVA], but both the 5 mg/kg group, $F(6,84)=6.01$, $p<0.00001$, and the 10 mg/kg group, $F(6,84)=9.48$, $p<0.00001$, differed from the saline group in time course (group × period interaction, 2 groups × 7 periods interaction for both comparisons).

*Time at Rest Without Elevating the Injected Paw*

There was little overall difference between 1 and 48 bar in the time spent at rest without elevating the injected paw (Fig. 2, lower panel; no significant pressure effect or pressure × dose interaction, 2 pressures × 4 doses × 7 periods ANOVA).

The overall effect of morphine was to increase the amount of time in this category [$F(3,56)=16.77$, $p<0.0001$ for dose effect, 2 pressures × 4 doses × 7 periods ANOVA], but at 1 bar, only the 10 mg/kg group had significantly higher values than the saline group [$F(1,14)=9.66$, $p<0.01$; 2 doses × 7 periods ANOVA]. At 48 bar there was a dose-related effect of morphine with each of the drug-treated groups showing significantly more time at rest than the saline group [2 doses × 7 periods ANOVA: $F(1,14)=5.02$, $p<0.05$ for 2.5 mg/kg, $F(1,14)=7.88$, $p<0.02$ for 5 mg/kg and $F(1,14)=37,96$, $p<0.00001$ for 10 mg/kg].

*Number of Paw-Jerks*

The number of paw-jerks was dose-relatedly reduced by morphine at both pressures but an interaction between pressure and dose was evident [Fig. 3; $F(3,56)=2.86$, $p<0.05$, 2 pressures × 4 doses × 7 periods ANOVA]. The scores did not differ between the two saline-treated groups (no significant group effect or group × period interaction, 2 groups × 7 periods ANOVA) but at 1 bar, significant effects of morphine started at the 5 mg/kg dose level, $F(1,14)=8.49$, $p<0.02$, while at 48 bar, the effects were significant also after the 2.5 mg/kg dose [$F(1,14)=7.86$, $p<0.02$, 2 doses × 7 periods ANOVA].

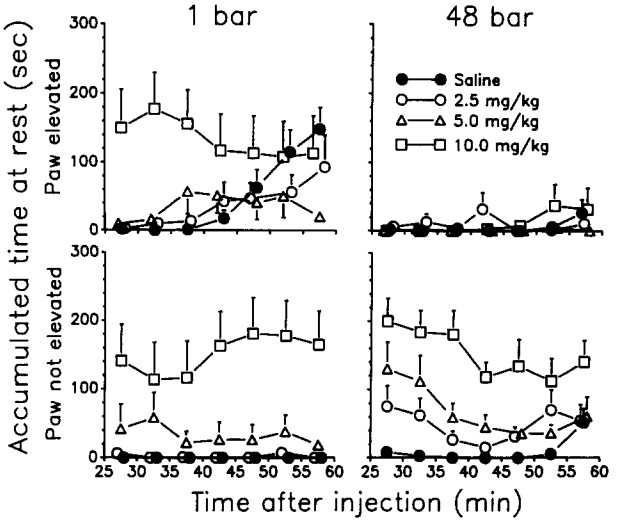


FIG. 2. Formalin test on rats at 1 or 48 bar ambient pressure: accumulated time at rest with or without the paw elevated. For further details, see legend to Fig. 1.

## DISCUSSION

This investigation of the behavior of rats in the formalin test shows that pressurization and morphine administration changes the amount of pain-related motor activity induced by formalin and alters the pattern of exhibited behavior. The number of paw-jerks was dose-dependently reduced by morphine both at 1 and 48 bar. While pressure per se did not significantly change this response, morphine appeared to be slightly more potent at 48 bar. Pressurization completely suppressed all other pain-related behavior directed towards the injected paw (licking, biting and shaking of the paw and clutching the paw tightly against the flank during locomotion) as well as resting with the paw elevated. The animals at pressure were also less inclined to protect

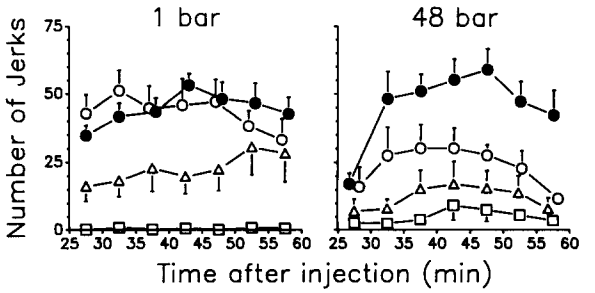


FIG. 3. Formalin test on rats at 1 or 48 bar ambient pressure: number of paw-jerks. The rats were given saline (filled circles) or morphine (open symbols; circles: 2.5 mg/kg, triangles: 5.0 mg/kg, squares: 10.0 mg/kg). Further details are given in legend to Fig. 1.

the injected paw during motor activity. Pressurization increased the amount of time spent in apparently normal motor activity.

Theoretically, freezing as a response to the stress of pressurization might have interfered with the pain-related behavior. Rats have previously been shown to freeze extensively in the formalin test after exposure to electrical shock (11). However, freezing was not observed in a pilot study under conditions identical to those of the present study (Berge et al., unpublished observations) and although not specifically scored in these experiments, would have been included in the normal resting category. Resting was increased by morphine but not by pressurization and was not predominant in the saline-treated groups.

It seems more likely that the decrease in most categories of pain-related behavior at pressure is related to an increase in background motor activity. Separate experiments under identical conditions have shown that without formalin injection, saline-treated rats spend more of their time resting than in the present experiments and the level of motor activity is significantly higher at 48 bar than at 1 bar. When active, the behavior does not resemble the pain-related activity seen after formalin [(13), Berge et al., unpublished observations]. The motor activity observed at 1 bar in the present study must, therefore, to a great extent have been induced by the injection of formalin. Supporting this assumption, nearly all the motor activity observed in saline-treated animals at 1 bar was classified as being pain-related. In contrast, the rats at 48 bar spent most of the observation time at activities not scored as pain-related. In these groups, the formalin-induced responses would be performed against a background of increased motor activity and possibly in competition with such activity. On this basis, it seems likely that general motor excitation, which is a well-documented effect of pressurization, may reduce the time spent in specific pain-related activity after formalin administration.

Other factors including diffuse noxious inhibitory controls (DNIC) (24,25) triggered by barotrauma to the ears, stress-induced analgesia and suppression of behavioral responses to pain, may also have affected the response pattern. Several studies have previously demonstrated stress-induced reductions of responses in the formalin test (2, 5, 11). We are at present investigating the possible contribution of these factors to the altered behavior. The observation that paw-jerking was not reduced at pressure, however, suggests that the pain sensitivity of the animals was unaltered.

In these experiments, the ambient temperature was adjusted as previously described in order to sustain normal body temperature in spite of the increased heat-loss caused by the thermal properties of helium (7). Measurement of tail skin temperature under identical ambient conditions has shown that at 48 bar, the temperatures are 3–3.5°C higher than at 1 bar, reflecting the difference in gas temperature (7). Studies in mice have shown that the response in the late phase of the formalin test is affected by environmental temperature and vasomotor tone of the paw skin (28). The effect seems to occur within a narrow range of temperatures near normal room temperature and it is doubtful that such mechanisms were important in the present experiment. The temperature difference would have tended to favor the inflammatory response in the 48-bar groups, and does not offer an explanation for the observed reductions in most pain-related behaviors.

Most of the behavior assumed to be pain-related was reduced in a dose-dependent manner after morphine. The notable exception was paw-elevation while resting, which was significantly increased in the 1-bar group after 10 mg/kg and was in fact the predominant category observed after this dose. In the saline group, the amount of time scored in this category showed a substantial increase during the second half of the session, at a time when licking, biting, shaking and clutching the paw towards the abdomen were greatly reduced. Thus, in the 1-bar condition, paw-elevation while at rest was exhibited at times when active responses indicative of pain were reduced or absent. At 48 bar, only negligible amounts of time were spent in this category. Since resting with the paw elevated only occurs in the absence of overt motor activity, a number of factors, including drug-induced sedation, habituation to the test situation or the absence of pain of sufficient strength to induce active responses directed towards the paw, favor its expression while the converse is the case for factors that cause increased motor activity. A previous study found that a low dose of morphine more than doubled the duration of paw elevation of rats while higher doses caused a dose-related reduction in the response (4). The study did not distinguish paw elevation during resting from paw elevation during activity, employed a weaker stimulus and differed from ours in several other methodological aspects, preventing a direct comparison of the results. However, it seems clear that inclusion of paw-elevation during resting as an indicator of pain may lead to paradoxical results.

In the literature, different criteria have been used in the formalin test, either singly or in combination. When rats are tested, paw-elevation is usually employed, either as sole criterion as in the study discussed above (4) or as one factor contributing to a pain index (10). Given the results of the present study, caution seems warranted when using this response, particularly if the time when the animals are at rest is included.

In mice, licking and biting on the injected paw is the predominant response and is usually taken as a single indicator of pain (17, 20, 27, 30). Licking and biting would be a component of focused, pain-related activity in the present study and our data are compatible with this category being sensitive to morphine. The fact that the behavior is inhibited by pressurization may, however, indicate susceptibility to nonanalgesic treatment effects.

In our experience, jerking movements of the injected paw are a consistent and characteristic response to formalin in the rat. The response may be observed during periods of inactivity as well as when the animals are active. This study indicates that the number of paw-jerks may be a robust and relatively sensitive indicator of pain, in line with the conclusion of a recent methodological study where the number of flinches, a response category defined slightly more widely than the paw-jerk category of the present study, appeared to be less variable than time spent licking (31). The use of a single criterion may, therefore, be adequate in the formalin test. The advantage of using a single, easily defined and objective criterion is obvious. However, comprehensive scoring as in the present study provides a more complete picture of the behavior of the animals and in our opinion, more data, particularly from pharmacological studies, are needed for a definite evaluation of the merits of different criteria and rating scales.

Since pressure significantly altered the overall response to formalin, any conclusions with regard to the interaction of pressure and morphine will have to be tentative. In the categories where comparable activity was observed at 1 and 48 bar, i.e., time in normal resting posture and the number of paw-jerks, morphine appeared to be somewhat more potent at elevated pressure. Previous studies have shown that the distribution and elimination of morphine remain unaffected by pressures as high as 71 bar (1). Pressure of similar magnitude to that used in the present study has been found to increase the proportion of dorsal horn nociceptive neurons maximally inhibited by a low dose of morphine (29), suggesting that pressure may interact directly with the action of opiates. An alternative possibility is that the pressurization procedure causes a stress-induced potentiation of

morphine analgesia. Such interactions have been reported in other paradigms (23).

Only a few other studies have addressed the possible interaction between elevated pressure and morphine but the results are inconclusive. One study reported unaltered analgesia in mice tested with the hot-plate method at a pressure of approximately 18 bar (15). However, the response latencies of the control subjects were significantly reduced at pressure, probably due to the elevated ambient temperature. Furthermore, forepaw lick, which is an unreliable indicator of pain in this test (6, 18, 32), was employed. In another study, pressurization to approximately 7 bar did not alter the morphine dose-response of rats exposed to electric shocks in an avoidance paradigm (8). It should be noted that the pressures applied were relatively low in these studies.

In conclusion, the responses exhibited by rats in the formalin test show varying degrees of sensitivity to pharmacological treatment and changes in the testing environment. While the paw-jerk response seems to be particularly robust and reliable, the analysis of several separate criteria may still be preferable, at least until more data on the significance of the different responses are available. The results provide tentative evidence against pressure-induced changes in pain sensitivity and pressure reversal of morphine analgesia.

ACKNOWLEDGEMENTS

This work was supported by the Norwegian Research Council for Science and the Humanities, Hyperbaric Medical Research Program. Technical assistance was provided by Ms. Torhild Fjordheim Sunde and editorial assistance by Dr. Hugh M. Allen.

## REFERENCES

1. Aanderud, L.; Bakke, O. M. Pharmacokinetics of antipyrine, paracetamol and morphine in rat at 71 ATA. Undersea Biomed Res. 10: 193–201; 1983.
2. Abbott, F. V.; Franklin, K. B. J.; Connell, B. The stress of a novel environment reduces formalin pain: possible role of serotonin. Eur. J. Pharmacol. 126:141–144; 1986.
3. Abbott, F. V.; Franklin, K. B. J.; Ludwick, R. J.; Melzack, R. Apparent lack of tolerance in the formalin test suggests different mechanisms for morphine analgesia in different types of pain. Pharmacol. Biochem. Behav. 15:637–640; 1981.
4. Abbott, F. V.; Melzack, R.; Leber, B. F. Morphine analgesia and tolerance in the tail-flick and formalin tests: dose-response relationships. Pharmacol. Biochem. Behav. 17:1213–1219; 1982.
5. Amir, S.; Amit, Z. The pituitary gland mediates acute and chronic pain responsiveness in stressed and non-stressed rats. Life Sci. 24: 439–448; 1979.
6. Berge, O.-G.; Fasmer, O. B.; Hole, K. Serotonin receptor antagonists induce hyperalgesia without preventing morphine antinociception. Pharmacol. Biochem. Behav. 19:873–878; 1983.
7. Berge, O.-G.; Garcia-Cabrera, I. Effects of ethanol on body temperature of rats at high ambient pressure. Pharmacol. Biochem. Behav. 39:37–41; 1991.
8. Curley, M. D.; Walsh, J. M.; Burch, L. S. Behavioral effects of morphine on free-operant avoidance under hyperbaric pressure. Pharmacol. Biochem. Behav. 12:413–417; 1980.
9. Dickenson, A. H.; Sullivan, A. F. Subcutaneous formalin-induced activity of dorsal horn neurones in the rat: differential response to an intrathecal opiate administered pre or post formalin. Pain 30: 349–360; 1987.
10. Dubuisson, D.; Dennis, S. G. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4:161–174; 1977.
11. Fanselow, M. S. Shock-induced analgesia on the formalin test: effects of shock severity, naloxone, hypophysectomy, and associative variables. Behav. Neurosci. 98:79–95; 1984.
12. Fasmer, O. B.; Berge, O.-G.; Hole, K. Changes in nociception after lesions of descending serotonergic pathways induced with 5,6-dihydroxytryptamine. Different effects in the formalin and tail-flick tests. Neuropharmacology 24:729–734; 1985.
13. Garcia-Cabrera, I.; Berge, O.-G. Pressure reversal of the depressant effect of ethanol on spontaneous behavior in rats. Pharmacol. Biochem. Behav. 29:133–141; 1988.
14. Garcia-Cabrera, I.; Berge, O.-G. Interaction of high pressure and a narcotic dose of ethanol on spontaneous behavior in rats. Pharmacol. Biochem. Behav. 37:577–581; 1990.
15. Greenbaum, L. J.; Evans, D. E. Morphine analgesia in mice exposed to a helium-oxygen atmosphere at 266 PSIG. Aerospace Med. 41:1006–1008; 1970.
16. Halsey, M. J.; Wardley-Smith, B. Pressure reversal of narcosis produced by anaesthetics, narcotics and tranquillisers. Nature 257:811–813; 1975.
17. Hunskaar, S.; Berge, O.-G.; Hole, K. Antinociceptive effects of orphenadrine citrate in mice. Eur. J. Pharmacol. 111:221–226; 1985.
18. Hunskaar, S.; Berge, O.-G.; Hole, K. A modified hot-plate test sensitive to mild analgesics. Behav. Brain Res. 21:101–108; 1986.
19. Hunskaar, S.; Berge, O.-G.; Hole, K. Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test. Pain 25:125–132; 1986.
20. Hunskaar, S.; Fasmer, O. B.; Hole, K. Formalin test in mice, a useful technique for evaluating mild analgesics. J. Neurosci. Methods 14:69–76; 1985.
21. Johnson, F. H.; Brown, D. E. S.; Marsland, D. A. Pressure reversal of the action of certain narcotics. J. Cell. Comp. Physiol. 20: 269–276; 1942.
22. Johnson, F. H.; Flagler, E. A. Hydrostatic pressure reversal of narcosis in tadpoles. Science 112:91–92; 1950.
23. Kelly, S. J.; Franklin, K. B. J. Evidence that stress augments morphine analgesia by increasing brain tryptophan. Neurosci. Lett. 44: 305–310; 1984.
24. Kraus, E.; Le Bars, D.; Besson, J.-M. Behavioral confirmation of "diffuse noxious inhibitory controls" (DNIC) and evidence for a role of endogenous opiates. Brain Res. 206:495–499; 1981.
25. LeBars, D.; Dickenson, A. H.; Besson, J.-M. Diffuse noxious inhibitory controls (DNIC). 1. Effects on dorsal horn convergent neurones in the rat. Pain 6:283–304; 1979.
26. Miller, K. W.; Paton, W. D. M.; Smith, R. A.; Smith, E. B. The pressure reversal of general anesthesia and the critical volume hypothesis. Mol. Pharmacol. 9:131–143; 1973.
27. Murray, C. W.; Porreca, F.; Cowan, A. Methodological refinements to the mouse paw formalin test: an animal model of tonic pain. J. Pharmacol. Methods 20:175–186; 1988.
28. Rosland, J. H. The formalin test in mice: the influence of ambient temperature. Pain 45:211–216; 1991.
29. Smallman, K.; Dickenson, A. H.; Halsey, M. J. Electrophysiological studies on the effects of opiates on dorsal horn nociceptive neurones at 51 atmospheres absolute. Pain 38:57–64; 1989.
30. Vaccarino, A. L.; Tasker, R. A. R.; Melzack, R. Systemic administration of naloxone produces analgesia in BALB/c mice in the formalin pain test. Neurosci. Lett. 84:103–107; 1988.
31. Wheeler-Aceto, H.; Porreca, F.; Cowan, A. The rat paw formalin test: comparison of noxious agents. Pain 40:229–238; 1990.
32. Woolfe, G.; MacDonald, A. D. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J. Pharmacol. Exp. Ther. 80:300–307; 1944.