Pharmacology Biochemistry & Behavior, Vol. 31, pp. 27–31. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Analgesia Induced by Continuous Versus Intermittent Cold Water Swim in the Rat: Differential Effects of Intrathecal Administration of Phentolamine and Methysergide

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Received 2 November 1987

ROCHFORD, J. AND J. L. HENRY. *Analgesia induced by continuous versus intermittent cold water swim in the rat: Differential effects of intrathecal administration of phentolamine and methysergide.* PHARMACOL BIOCHEM BEHAV 31(1) 27–31, 1988.—Continuous cold water swim produces analgesia that is partially mediated by a noradrenergic mechanism, but is independent of both serotonergic and opioid systems. On the other hand, intermittent cold water swim elicits analgesia which is partly mediated by an opioid mechanism; the contribution of the monoamines to the production of this analgesia is not known. Therefore, the present study was done to determine whether intermittent cold water swim is also mediated by noradrenergic and/or serotonergic substrates. Prior to either continuous (3.5 min) or intermittent (10 sec in, 10 sec out for 6 min) cold water (4°C) swim, male Sprague-Dawley rats (225–250 g) were administered either the noradrenergic receptor blocker phentolamine (30 μg), the serotonergic blocker methysergide (30 μg) or artificial cerebrospinal fluid to the fifth lumbar vertebral spinal level via chronic intrathecal catheters. Phentolamine significantly attentuated the analgesia resulting from both continuous and intermittent cold water swim. Methysergide attenuated intermittent cold water swim analgesia, but was without effect on continuous cold water swim analgesia. Phentolamine, but not methysergide, also attenuated continuous footshock- (2.5 mA for 3 min) induced analgesia. The similarity between the effects of phentolamine and methysergide on continuous footshock and continuous cold water swim analgesia suggests that the effects of these drugs on cold water swim analgesia are not attributable to changes in thermoregulation. These results suggest that a spinal noradrenergic mechanism is involved in the mediation of both forms of cold water swim analgesia, whereas a spinal serotonergic mechanism is involved in only intermittent cold water swim analgesia.

Noradrenaline Serotonin Stress-induced analgesia Opioid Nonopioid Intrathecal

CONTINUOUS cold water swim (3.5 min) produces a profound, long-lasting increase in pain threshold in the rat. Evidence has implicated noradrenaline in the mediation of continuous cold water swim analgesia in that systemic administration of clonidine, an alpha adrenergic receptor agonist, and desipramine, a noradrenaline reuptake blocker, both potentiate the effect [8,9]. Conversely, current evidence suggests that serotonin is not involved in the mediation of continuous cold water swim analgesia; administration of para-chlorophenylalanine, a potent tryptophan hydroxylase inhibitor, is without effect [7]. In addition, opioid peptides do not appear to be involved because cold water swim analgesia is only partly attenuated by high doses of the opiate receptor blocker naloxone and does not display cross tolerance with morphine-induced analgesia [4,6].

Modification of the temporal parameters of cold water swim exposure alters the neurochemical substrates mediating the analgesia. Specifically, the analgesia elicited by exposure to intermittent (10 sec in, 10 sec out for 6 min), rather than continuous, cold water swim is attenuated by opiate receptor blockade and displays cross tolerance to morphine [15–17]. These results suggest that the manner in which a stressor, in this case cold water swim, is applied critically determines whether opioid substrates are activated (see also [18,19]).

Because alterations of the temporal parameters of swim administration determine the opioid/nonopioid nature of the

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resultant analgesia, we assumed that the influence of noradrenaline and serotonin might also be affected by this manipulation. Consequently, the present experiment examined the effects of the administration of the noradrenergic and serotonergic receptor antagonists, phentolamine and methysergide, on continuous and intermittent cold water swim analgesia.

A second goal of the present experiment was to determine the importance of spinal noradrenergic and serotonergic mechanisms in cold water swim analgesia. In previous work the importance of these transmitters was assessed by the systemic administration of drugs that alter their function [7–9]. This route of administration does not dissociate the importance of spinal versus supra-spinal sites of action. Therefore, in the present experiment phentolamine and methysergide were applied directly to the spinal cord via chronic intrathecal catheters.

Finally, because exposure to cold water produces profound hypothermia [5], it was possible that any observed effects of phentolamine or methysergide on cold water swim analgesia may have been attributable to changes in thermoregulation, as opposed to pain reactivity. In order to control for this possibility, we examined the effects of intrathecal administration of phentolamine and methysergide on analgesia induced by a stressor that should not produce a pronounced hypothermia, namely, continuous footshock.

METHOD

Subjects

Experimentally naive, male albino Sprague-Dawley rats (Charles River Canada, Inc., St. Constant, Quebec), weighing 225–250 g on arrival at our laboratory, were used. The rats were individually housed and provided with free access to food and water. They were maintained on a 12:12 hr light/dark cycle (lights on 7:00–19:00). All experimental procedures were conducted during the light component of the cycle.

Surgery

Each rat was implanted with a chronic intrathecal catheter (Intramedic PE10). Briefly, the animals were anaesthetized with chloral hydrate (300 mg/kg) and the catheter was inserted through an incision made in the dura at the atlanto-occipital junction. The catheter was inserted so that the inner tip lay at the fifth lumbar vertebral level, and then fixed with dental cement to a stainless steel screw imbedded in the skull. Rats displaying any neurological deficit following surgery were excluded from the study. The remaining rats were given one week to recover.

The correct position of the catheter tip was verified in two ways. First, prior to the start of the experiment, 25 μl of Xylocaine (10 mg/kg) were administered intrathecally. Only those rats showing a reversible sensory (lack of response to noxious tail pinch) and motor blockade (dragging of at least one of the hind legs) were used in the experiment. Second, after completion of the experiment the rats were sacrificed, and the lumbar spinal cord was exposed by laminectomy. The position of the catheter tip was visually confirmed by injection of 20 μl of Evans Blue dye.

Test for Analgesia

Analgesia was assessed by the tail-flick test. Each rat was placed into a plastic restrainer covered with a black cloth to reduce visual stimuli. Nociceptive threshold was measured as the reaction time to movement of the tail from above a noxious radiant heat source (a projector bulb) focussed 5 cm proximal to the tip of the tail which was blackened to promote heat absorption. Reaction time was mechanically recorded to the nearest hundredth of a second. The bulb intensity was adjusted to produce tail-flick latencies between 4 and 5 sec under control conditions. Trials were automatically terminated after 12 sec to avoid tissue damage. Prior to the start of the experiment the rats were acclimatized to the restrainers (30 min/day for 3–4 days) to minimize the effects of restraint stress.

Procedure

The effects of phentolamine and methysergide on continuous and intermittent cold water swim analgesia. The rats were randomly divided into six groups of eight animals each. Three of the groups were exposed to continuous cold water swim, which consisted of a 3.5 min swim in water at 4°C. The remaining three groups were submitted to intermittent cold water swim, consisting of 10 sec in the water followed by 10 sec out of the water for 6 min. Within each stress condition, one of the groups was administered intrathecally 20 μl of artificial cerebrospinal fluid (CSF; aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 2.0 mM $MgCl_2$ and 1.4 mM $CaCl_2$; phosphate buffered, pH 7.33). The second group received phentolamine mesylate (Ciba-Geigy), and the third group received methysergide bimaleate (Sandoz Inc.). Phentolamine and methysergide were dissolved in artificial CSF; 30 μg were injected in a 10 μl volume followed by 10 μl of CSF to flush the catheter. This dose was selected because it has been used by previous investigators to examine the influence of spinal noradrenergic and serotonergic mechanisms on antinociception (e.g., [14,21]).

For each rat three tail-flick tests were conducted at 5 min intervals; the mean of these three trials was calculated and constituted the baseline tail-flick latency. The rat was then administered its designated intrathecal solution, and stressed 15 min later. Poststress tail-flick latencies were determined 30 and 60 min following termination of the stressor.

The effects of intrathecal phentolamine and methysergide in the absence of stress. Each rat was exposed to cold water swim stress once. However, 3–4 days after the stress day some rats were used to determine the effects of intrathecal administration of phentolamine and methysergide on pain threshold in the absence of stress. The baseline tail-flick latency was determined as on the stress day. The rats were then administered either CSF, phentolamine or methysergide (same injection as administered on the stress day). Tail-flick tests were conducted 50 and 80 min after injection. These times corresponded to the time the rats were tested following injections on the stress day.

The effects of intrathecal phentolamine and methysergide on continuous footshock-induced analgesia. In this experiment 21 rats not used in the previous experiments were randomly divided into three groups of 7. Following determination of baseline tail-flick latencies (mean of 3 trials spaced 5 min apart), one group was intrathecally administered methysergide. Doses and volumes were identical to those employed in the previous experiments. Ten minutes later each rat was placed into a wooden chamber, and 2.5 mA footshock was applied continuously for 3 min through the steel grid floor of the chamber. Postshock tail-flick trials were conducted at 1, 3, 5, 7, 9, 11, 13 and 15 min following shock termination.

TABLE 1

MEAN (±SEM) PERCENT OF MAXIMUM POSSIBLE EFFECT ON REACTION TIME IN THE TAIL FLICK TEST FOR GROUPS INTRATHECALLY ADMINISTERED ARTIFICIAL CEREBROSPINAL FLUID (CSF), 30 μg PHENTOLAMINE (PHENT) OR 30 μg METHYSERGIDE (METHY) 15 MIN PRIOR TO CONTINUOUS (CON) OR INTERMITTENT (INT) SWIM IN COLD WATER AT 4°C (N=8 PER GROUP)

	Time After Swim	
Group	30 Min	60 Min
CON-CSF	43.69 ± 11.48	15.82 ± 7.01
CON-PHENT	8.85 ± 5.79	2.20 ± 3.27
CON-METHY	32.41 ± 4.49	18.32 ± 2.84
INT-CSF	86.35 ± 9.35	47.77 ± 11.87
INT-PHENT	44.80 ± 9.50	23.91 ± 12.10
INT-METHY	31.38 ± 5.90	10.05 ± 3.50

Data Analysis

Tail-flick latencies were transformed to the percentage of the maximum possible effect (%MPE), according to the following formula:

$$\%MPE = [(TFL - BL)/(12 - BL)] \times 100$$

where TFL=poststress/postinjection tail-flick latency, BL=mean baseline latency, and 12=cut-off latency. Separate split-plot analyses of variance (ANOVAs) were conducted on the data from each experiment (cold water swim, no stress, continuous footshock). Pairwise comparisons between groups were conducted with Tukey's Wholly Significant Difference Test [20]. The level of significance adopted was $p<0.05$.

RESULTS

The Effects of Intrathecal Phentolamine and Methysergide on Cold Water Swim Analgesia

Table 1 shows the effects of intrathecal administration of CSF phentolamine and methysergide on continuous and intermittent cold water swim-induced analgesia. Exposure to both forms of swim produced a marked analgesic effect; the magnitude of the effect was significantly greater in the intermittent swim condition, $F(1,42)=13.82$, $p<0.001$. Both analgesic responses dissipated over trials, $F(1,42)=38.39$, $p<0.001$. Intrathecal administration of phentolamine and methysergide differentially attenuated the analgesia resulting from continuous and intermittent cold water swim, as was confirmed by a significant intrathecal injection × type of swim interaction, $F(2,42)=5.39$, $p<0.01$. Tukey's tests revealed that intrathecal administration of phentolamine significantly reduced continuous cold water swim analgesia. Collapsed across trials, the mean %MPE (±SEM) for CSF-treated animals was 29.76% (±7.43), while that for the phentolamine group was 5.51% (±3.33). In contrast, the mean %MPE for animals administered methysergide (25.36±3.15%) did not differ significantly from the group administered CSF.

Tukey's tests also revealed that the means for the groups administered phentolamine (34.35±7.91%) and methysergide (20.72±4.31%) and exposed to intermittent cold water swim were significantly lower than the mean for the CSF-treated group (67.06±8.84%). These results suggest that methysergide was a more potent inhibitor of intermittent cold water swim analgesia than phentolamine. This conclusion, however, was not confirmed statistically, as the difference between these two groups was not significant.

The Effects of Phentolamine and Methysergide in Nonstressed Animals

Intrathecal administration of both methysergide and phentolamine in the absence of stress tended to lower pain threshold relative to CSF-treated animals (data not shown). However, this trend was not statistically confirmed as neither the main effect for intrathecal injection, $F(2,21)=3.13$, nor the intrathecal injection × trials interaction, $F(2,21)=1.98$, were significant, $ps>0.05$.

The Effects of Phentolamine and Methysergide on Continuous Footshock Analgesia

The ANOVA conducted on the data from the continuous footshock experiment revealed a significant main effect for trials, $F(7,126)=8.56$, $p<0.001$, indicating that the magnitude of the analgesic response induced by continuous footshock dissipated over trials. The main effect for type of injection was also significant, $F(2,18)=8.01$, $p<0.005$, although the type of injection × trials interaction was not significant, $F(14,126)=0.50$, $p>0.05$. Tukey's tests revealed that the mean %MPE for the group administered phentolamine (3.36±2.37%) was significantly lower than the mean %MPE for the CSF-treated group (55.03±4.13%). In contrast, the mean %MPE for the methysergide-treated group (30.47±5.33%) did not differ significantly from the CSF group ($p>0.05$).

DISCUSSION

The results from the cold water swim are consistent with the work of Bodnar and associates indicating that continuous cold water swim analgesia is mediated by noradrenergic, but not serotonergic, mechanisms [7–9]. Intrathecal administration of phentolamine, but not methysergide, significantly attenuated the effect.

More interesting were the results from the intermittent swim condition, where both receptor antagonists significantly attenuated the analgesia. Thus, it would appear that intermittent cold water swim analgesia, in addition to being mediated by endogenous opioids [15–17], is mediated by both noradrenergic and serotonergic mechanisms. The finding that phentolamine and methysergide differentially affect continuous and intermittent cold water swim analgesia is consistent with the suggestion that the temporal parameters of stress administration influence which neurochemical mechanisms will be activated (for review, see [27]).

We also found that intrathecal administration of phentolamine, but not methysergide, significantly attenuated the analgesia elicited by continuous footshock, a pattern which parallels that obtained with continuous cold water swim. This similarity suggests that the effects of phentolamine and methysergide on cold water swim analgesia are attributable to the influence of these drugs on antinociceptive mechanisms, and not to their effects on thermoregulation. This conclusion is reinforced by two additional lines of evidence. First, manipulation of monoaminergic function alters continuous cold water swim analgesia in the flinch-jump test

[7–9], a nociceptive test that does not involve exposure to a thermal noxious stimulus. Second, the analgesic and hypothermic effects of continuous cold water swim are dissociable. For example, continuous cold water swim analgesia, but not hypothermia, is altered by hypophysectomy [2] and repeated exposure to cold water swims [5].

The fact that phentolamine and methysergide were administered intrathecally suggests that they attentuate cold water swim-induced analgesia by inhibiting spinal antinociceptive mechanisms, most probably the descending noradrenergic and serotonergic fibers which originate in several nuclei in the rostral medulla [1,13]. The incomplete blockade of intermittent cold water swim analgesia by phentolamine and methysergide suggests that at least one other substrate, possibly opioid in nature, is also activated by this type of stress. On the other hand, the near complete reversal of continuous cold water swim analgesia by intrathecal phentolamine implies that a spinal noradrenergic mechanism is the sole mediator of this type of analgesia. This conclusion, however, must be viewed cautiously, since previous work has implicated acetylcholine [22,26], dopamine [3,10], gonadal steroids [23,24] and vasopressin [11,30] in the neurochemical mediation of continuous cold water swim analgesia. One possibility is that the spinal noradrenergic mechanism implicated by the results of the present experiment may be the final common pathway upon which each of these neurochemicals exerts (either directly or indirectly) its effects. Further work will be required in order to determine the relative contributions of these diverse neurochemicals, as well as the nature of their possible interactions, in the mediation of this form of stress-induced analgesia.

The results from the present experiment suggest that noradrenaline and serotonin both mediate opioid stress-induced analgesia, whereas only noradrenaline mediates nonopioid analgesia. These conclusions, however, have not been consistently supported by studies investigating the neurochemical mediation of opioid/nonopioid analgesia induced by stressors other than cold water. For example, the influence of noradrenergic and sertonergic mechanisms on opioid and nonopioid shock-induced analgesia is, at present, ambiguous. Inhibition of monoaminergic function has been reported to attenuate [12, 28, 29, 31, 32], to potentiate [12, 25, 28], or to have no effect upon [12,31] opioid and/or nonopioid shock-induced analgesia. The reason for these divergent results is, at present, unclear. They may reflect differences in experimental protocol, such as the parameters of shock administration, of the type of test used to measure analgesia, or differences in the route of administration of monoaminergic inhibitors.

It would appear, therefore, that the relationship between the monoamines and opioid/nonopioid stress-induced analgesia is relatively complex. The contribution of the monoamines to stress-induced analgesia appears to vary both between and within stressors; quantitative and/or qualitative differences in the application of stress may critically influence the nature of the neurochemical mediation of the analgesia. Consequently, it may be inadvisable to attempt to equate different stressors simply on the basis of whether or not they activate opioid mechanisms, because this may be the only similarity the two stressors share.

In conclusion, the results from the present study suggest that noradrenaline mediates the nonopioid analgesia induced by continuous cold water swim, whereas both noradrenaline and serotonin are involved in the opioid analgesia observed following intermittent cold water swim.

ACKNOWLEDGEMENTS

Supported by Grant MA-5891 from the Medical Research Council of Canada to J.L.H. J.R. was supported by a Fonds pour la formation de Chercheurs et L'Aide à la Recherche postdoctoral fellowship.

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