Pharmacology Biochemistry & Behavior, Vol. 33, pp. 261–263. Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

BRIEF COMMUNICATION

MIF-1 and Tyr-MIF-1 Fail to Alter Benzodiazepine-Induced Hypothermia

ABBA J. KASTIN,[1] LAWRENCE G. MILLER AND DEBRA S. SCHWARTZENBURG

VA Medical Center, LSU Medical Center, University of New Orleans and Tulane University School of Medicine

Received 6 October 1988

KASTIN, A. J., L. G. MILLER AND D. S. SCHWARTZENBURG. *MIF-1 and Tyr-MIF-1 fail to alter benzodiazepine-induced hypothermia.* PHARMACOL BIOCHEM BEHAV **33**(1) 261–263, 1989.—Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) and MIF-1 (Pro-Leu-Gly-NH_2) can increase GABA-stimulated benzodiazepine binding in brain tissue and can block the hypothermia induced by several other compounds. Since benzodiazepines can also cause hypothermia, colonic temperatures were measured in mice after administration of chlordiazepoxide (CDP) and these two brain peptides. In several experiments involving different doses and times of administration of CDP, MIF-1, and Tyr-MIF-1, there were no significant effects of the peptides in altering the reliable decrease in colonic temperature induced by the benzodiazepine. The results indicate that the interaction of Tyr-MIF-1 and MIF-1 with benzodiazepines does not involve thermoregulation.

MIF-1 Tyr-MIF-1 Naloxone Peptides Temperature Chlordiazepoxide Benzodiazepine

THE endogenous (2) brain peptide Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) can increase benzodiazepine receptor binding after peripheral administration in mice (4), probably by a direct action at the GABA receptor. In addition, this tetrapeptide can modulate binding at the putative chloride channel site on the receptor, as well as overall receptor function (5–7).

Although MIF-1 (Pro-Leu-Gly-NH_2) by itself does not affect body temperature (8), it does interact with other substances to alter thermoregulation. The hypothermia induced by chlorpromazine (8), reserpine (3), and β-endorphin (9) are blocked by MIF-1.

Benzodiazepines can also cause hypothermia, but the mechanism for this effect is still unknown (1). Because of the interactions of Tyr-MIF-1 and MIF-1 with benzodiazepine binding and with drug-induced hypothermia, we studied the effects of these peptides on the hypothermia induced by the benzodiazepine chlordiazepoxide (CDP).

METHOD

Materials

CDP (Librium) was kindly provided by Hoffmann-La Roche Inc. (Nutley, NJ) and naloxone by Dupont (Wilmington, DE). MIF-1 and Tyr-MIF-1 were purchased from Bachem AG (Bubendorf, CH).

Animals

Male ICR mice weighing 30–45 g were purchased from Charles River Laboratories (Wilmington, MA). They were maintained on a 12:12 hr light-dark cycle with lights on at 6 a.m. Testing was performed between 8 a.m. and 12 p.m. Food and water were freely available at all times.

Procedure

Mice were injected intraperitoneally (IP) with CDP. Fifteen min later, they were injected IP with the test compound or diluent (0.9% NaCl, 0.01 M acetic acid), 10 ml/kg. Fifteen min after injection of the test substance (30 min after injection of the CDP), a rectal probe was inserted 2.5 cm into each mouse and temperature recorded (Sensortek Inc., Clifton, NJ).

Statistics

Data were compared by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test.

RESULTS

Experiment 1

The dose of 80 mg/kg CDP used by Chan *et al.* (1) was

[1]Requests for reprints should be addressed to A. J. Kastin, VA Medical Center, 1601 Perdido St., New Orleans, LA 70146.

followed 15 min later by a dose of 1 mg/kg MIF-1 or diluent. This dose of MIF-1 was found to reverse the hypothermia induced by chlorpromazine (8) and β-endorphin (9). There was no significant difference in mean colonic temperature in the mice receiving MIF-1 or diluent (34.8 ± 0.3 vs. 34.9 ± 0.5°C, n = 10 each). Since it was possible that the dose of CDP was too strong for the effect of MIF-1 to be exerted, we attempted in the next two experiments to find the smallest effective doses of CDP and peptide.

Experiment 2

The effects of four doses of CDP were compared with those of diluent. ANOVA showed a highly significant effect of treatment, $F(4,25) = 14.32$, $p<0.001$. A dose-response relationship was found, with the highest dose of 80 mg/kg resulting in the lowest temperatures (34.8 ± 0.3°C, $p<0.0001$), followed by the dose of 60 mg/kg (35.6 ± 0.4°C, $p<0.0001$), and 40 mg/kg (36.2 ± 0.4°C, $p<0.001$). The lowest dose tested, 20 mg/kg IP, showed a tendency in the same direction (37.2 ± 0.4°C, $p=0.08$) as compared with the control (38.1 ± 0.3°C).

Experiment 3

The lowest dose (40 mg/kg) of CDP causing a statistically significant reduction in colonic temperature in Experiment 2 was selected. MIF-1 and Tyr-MIF-1 were injected in doses of 0, 0.1, 1, and 10 mg/kg, at least 6 mice/group. Although CDP again resulted in a significant hypothermia (36.3 ± 0.4 vs. 38.6 ± 0.2°C, $p<0.0001$), no dose of peptide significantly affected the CDP-induced hypothermia. Although not statistically significant, the lowest mean temperature was seen after CDP + 0.1 mg/kg MIF-1 and the highest mean temperature was seen after CDP + 0.1 mg/kg Tyr-MIF-1. These doses of peptide were selected for the next experiment.

Experiment 4

A lower dose of CDP (30 mg/kg) was followed by 0.1 mg/kg MIF-1, 0.1 mg/kg Tyr-MIF-1, 0.1 mg/kg naloxone, or diluent in groups of 6 mice each. CDP lowered basal colonic temperature from 38.3 ± 0.2°C to 36.5 ± 0.3°C, $p<0.0001$, but MIF-1, Tyr-MIF-1, and naloxone did not significantly alter the hypothermia. Opposite to the previous experiment, the 0.1 mg/kg dose of Tyr-MIF-1 resulted in the lowest temperature and the 0.1 mg/kg dose of MIF-1 in the highest temperature, but again these findings were not statistically significant.

Experiment 5

Since there was a tendency for the only dose of Tyr-MIF-1 tested in Experiment 4 to lower colonic temperature, the low dose of CDP (30 mg/kg) was tried with several doses (0, 0.1, 1, and 10 mg/kg) of Tyr-MIF-1 and MIF-1. CDP lowered basal temperature from 38.7 ± 0.1°C to 35.5 ± 0.2°C, $p<0.0001$. There was no indication of any enhancement of the hypothermic effect of CDP as shown by the nonsignificant tendency for the lowest temperatures to occur in the two groups receiving diluent.

Experiment 6

In this experiment, 0.1 mg/kg dose of Tyr-MIF-1 or diluent were injected 15 min before, at the same time, or 5 min after 40 mg/kg CDP. At 30 min, the CDP lowered basal body temperature from 39.4 ± 0.1 to 36.1 ± 0.4°C in the group injected with diluent 15 min before the CDP ($p<0.00001$), from 39.2 ± 0.2 to 36.4 ± 0.3°C in the group injected with diluent at the same time as CDP ($p<0.00001$), and from 39.0 ± 0.2 to 36.2 ± 0.3°C in the group injected with diluent 5 min after the CDP ($p<0.00001$). The temperature recorded in each group (n = 6) every 30 min for the 3 hr after injection of the CDP (Fig. 1) revealed no significant effect of treatment (peptide vs. diluent) or time at which the treatment was administered in relation to the CDP. At no single time for any of the sequences were the effects of Tyr-MIF-1 on colonic temperature different from those of the diluent by Duncan's Multiple Range Test.

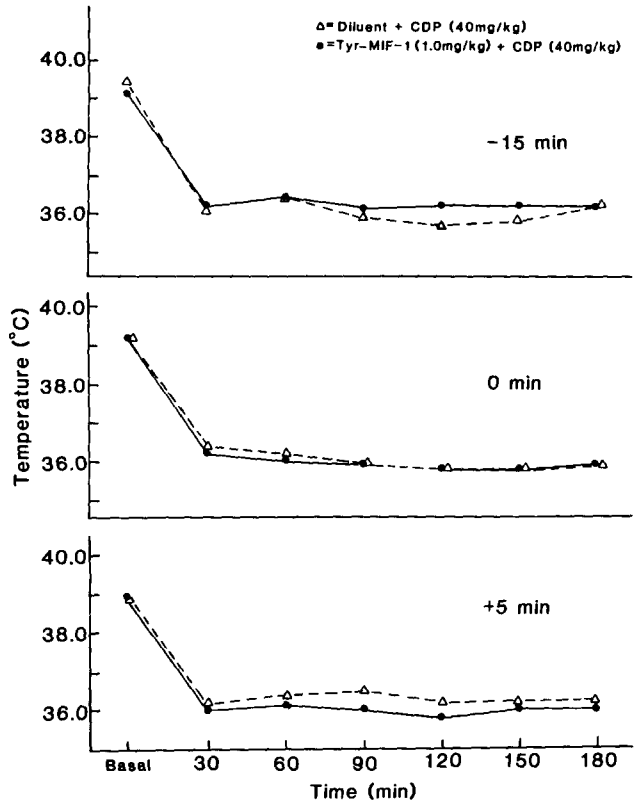

FIG. 1. Mean colonic temperature in Experiment 6 after Tyr-MIF-1 (0.1 mg/kg, IP) or diluent injected 15 min before (−15′), at the same time (0′), or 5 min after (+5′) CDP (40 mg/kg, IP). The previous experiments involved injections of various doses of MIF-1 and Tyr-MIF-1 15 min after the CDP.

DISCUSSION

Despite evidence for an interaction of MIF-1 and Tyr-MIF-1 with the benzodiazepine receptor (4,5) and with hypothermia caused by other compounds (3, 8, 9), no significant change in CDP-induced hypothermia was found after administration of these brain peptides. By contrast, the benzodiazepine antagonist Ro15-1788 markedly blocked the hypothermic effect of CDP (80 mg/kg, IP) at 30 min (1).

It is possible that an interaction of MIF-1 and Tyr-MIF-1 with the hypothermic effect of CDP might be found under different conditions, but the times and doses used were in the range previously found effective in other paradigms involving these compounds. Although the increased benzodiazepine binding induced by these peptides in several parts of the brain was not found to be significant in the hypothalamus (4), the relationship of this finding to the present results is not known. If MIF-1 and Tyr-MIF-1 affect the actions of benzodiazepines as they do binding, this interaction does not seem to involve hypothermia.

ACKNOWLEDGEMENTS

Supported by the VA and NIH (DA-05258).

REFERENCES

1. Chan, A. W. K.; Langan, M. C.; Schanley, D. L.; Penetrante, M. L.; Leong, F. W.; Aldrich-Castanik, L. Differential effects of Ro15-1788 in actions of chlordiazepoxide and ethanol. Pharmacol. Biochem. Behav. 29:315–320; 1988.
2. Horvath, A.; Kastin, A. J. Isolation of Tyr-MIF-1 from bovine brain tissue. J. Biol. Chem. 264:2175–2179; 1989.
3. Kastin, A. J.; Honour, L. C.; Coy, D. H. Effects of MIF-1 and three related peptides on reserpine-induced hypothermia in mice. Pharmacol. Biochem. Behav. 15:983–985; 1981.
4. Miller, L. G.; Kastin, A. J.; Greenblatt, D. J. Tyr-MIF-1 augments benzodiazepine receptor binding in vivo. Pharmacol. Biochem. Behav. 28:521–524; 1987.
5. Miller, L. G.; Kastin, A. J. MIF-1 and Tyr-MIF-1 augment GABA-stimulated benzodiazepine receptor binding. Peptides 8:751–755; 1987.
6. Miller, L. G.; Kastin, A. J.; Roy, R. B. Effects of Tyr-MIF-1 and MIF-1 at $GABA_A$ receptor chloride channel site. Brain Res. Bull. 19:743–745; 1987.
7. Miller, L. G.; Kastin, A. J.; Roy, R. B. MIF-1 and Tyr-MIF-1 augment muscimol-stimulated chloride uptake in mouse cortical synaptoneurosomes. Brain Res. Bull.; submitted.
8. Yehuda, S.; Kastin, A. J. Interaction of MIF-1 or α-MSH with D-amphetamine of chlorpromazine on thermoregulation and motor activity of rats maintained at different ambient temperatures. Peptides 1:243–248; 1980.
9. Yehuda, S.; Kastin, A. J.; Coy, D. H. Antagonistic actions of MIF-1 on the hypothermia and hypomotility induced by β-endorphin or morphine. Int. J. Neurosci. 11:317–320; 1980.