Pharmacology Biochemistry & Behavior, Vol. 40, pp. 129–132. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Production of Hypothermia in the Guinea Pig by a Kappa-Agonist Opioid Alone and in Combination With Chlorpromazine

M. W. ADLER,[1] E. BRADLEY, R. MARTINEZ AND E. B. GELLER

Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140

Received 29 October 1990

ADLER, M. W., E. BRADLEY, R. MARTINEZ AND E. B. GELLER. *Production of hypothermia in the guinea pig by a kappa-agonist opioid alone and in combination with chlorpromazine.* PHARMACOL BIOCHEM BEHAV **40**(1) 129–132, 1991.—In rats, kappa opioids decrease body temperature and the combination of a selective kappa agonist with chlorpromazine induces a profound hypothermia. Because of the greater density of kappa-opioid receptors and increased ratio of kappa to mu in the guinea pig, the actions of these drugs on body temperature were compared in this species. Groups of young adult, male Hartley guinea pigs were injected SC with trans-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)-cyclohexyl)-benzeneacetamide methanesulfonate, hydrate (U50,488H; 20–80 mg/kg) and chlorpromazine (2.5–5 mg/kg), either alone or in combination. Rectal temperatures were measured over a 5-h period. U50,488H produced a dose-related decrease in temperature, with a mean maximum drop of approximately 7°C. The maximum decrease with chlorpromazine was approximately 1.6°C. When doses at the lower end of the range for each drug were given together, the combined effect was greater than that expected from the individual drugs. Both the peak effect and the duration of the hypothermia appeared to be potentiated. At the highest dose combination only an additive effect was seen. Compared to the rat, the hypothermic effect of the kappa agonist alone is much greater in the guinea pig. The potentiation between the two drugs, however, is greater in the rat.

Kappa receptor U50,488H Opioid Chlorpromazine Body temperature Hypothermia Guinea pig

THE opioids are known to produce changes in body temperature (T_b) that are dependent on a variety of factors such as species, strain, restraint, ambient temperature, dose, and route of drug administration (2, 3, 5). Earlier work from our laboratory led us to postulate that a two-receptor system was involved in the actions of opioids on T_b in the rat, that the opioid systems played a role in thermoregulation and that the mu system was hyperthermic and the kappa system was hypothermic (8,9). Much of this work was dealt with in a recent review (3).

Chlorpromazine is a phenothiazine antipsychotic that is known to lower body temperature in several species (7). Its use in combination with other drugs to make a "lytic cocktail" was first proposed in 1951 by Laborit and Huguenard as a means of enhancing whole-body cooling for surgical procedures (13). In addition to our findings with the opioids and opioid antagonists per se, we reported (1,4) that the combination of chlorpromazine and the highly selective kappa agonist, U50,488H, produced a profound hypothermia in the rat that amounted to as much as 11°C and lasted for up to 24 h after a single administration at an ambient temperature of 20°C. The effect appeared to be a true potentiation (greater than additive) of the actions of the individual drugs, and the hypothermia gradually diminished over the 24-h time period with full recovery of the animal. Naloxone could partially antagonize the hypothermia. In order to determine more about the generality of the kappa-induced hypothermia and about the potentiation of the effect by chlorpromazine, we decided to test these drugs in the guinea pig because this species has a much greater number and density of kappa-opioid receptors in the central nervous system than the rat (14). In a study with guinea pigs, Kandasamy and Williams (12) reported that a series of opioids and opioid peptides produced hyperthermia when given ICV. As no kappa-selective opioid (e.g., dynorphin A_{1-17} or U50,488H) was tested, conclusions cannot be drawn from this report regarding the actions of kappa receptors relative to T_b. We thus postulated that the dose-response curve for the hypothermia produced by a selective kappa agonist alone would be shifted to the left in the guinea pig as compared to the rat and that we would again see a potentiation when chlorpromazine and the kappa agonist were used simultaneously.

METHOD

Subjects were young adult, male Hartley guinea pigs weighing 350–400 g. They were housed in groups of 5 to a cage in animal quarters maintained at 22 ± 2°C and approximately 50% relative humidity for at least 1 week. Lights were on from 0700 to 1900 h. Food and water were available ad lib except during testing. The animals were kept in individual cages in a Hotpack

[1]Requests for reprints should be addressed to Dr. Martin W. Adler, Department of Pharmacology, Temple University School of Medicine, 3420 N. Broad St., Philadelphia, PA 19140.

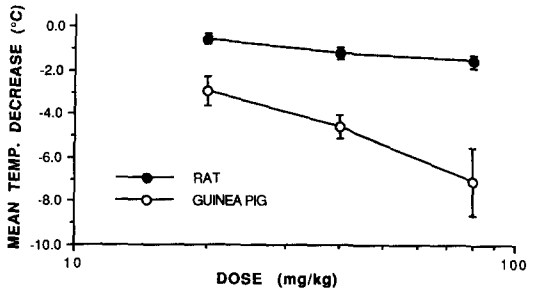

FIG. 1. Dose-response for U50,488H (20–80 mg/kg, SC) on body temperature of guinea pigs and rats.

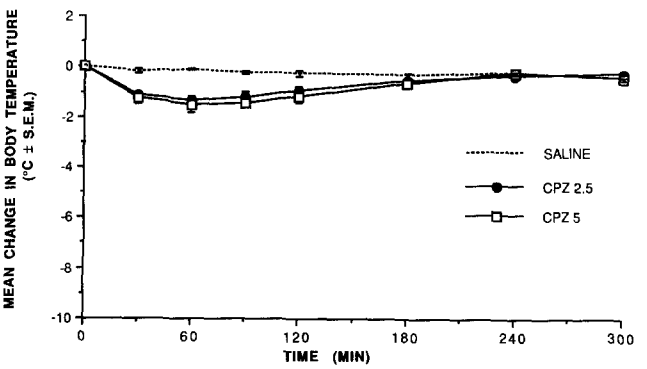

FIG. 2. Time-dependent effect of chlorpromazine (2.5 or 5 mg/kg, SC) on body temperature in guinea pigs. N = 5–6 per group.

environmental room for the entire duration of the test. All experiments began at approximately 0900 h and were conducted in an ambient of 20 ± 0.3°C and 55 ± 5% relative humidity. After a 30-min adaptation period, body core temperatures were recorded from a thermistor probe inserted 7 cm into the rectum. The guinea pig rested on the forearm of the technician with its head buried in the crook of the arm; no restraint was required during the measurement. Temperatures were read from a digital display thermometer. The initial 3 readings were taken at 30-min intervals; the first reading was discarded to allow for adaptation and the second and third were averaged for a baseline.

Groups of 5–7 animals were then injected SC with 20, 40, or 80 mg/kg of U50,488H, alone or in combination with 2.5 or 5 mg/kg of chlorpromazine. U50,488H ([trans-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)-cyclohexyl)-benzeneacetamide methanesulfonate, hydrate]; U) was kindly supplied by The Upjohn Company, Kalamazoo, MI. Chlorpromazine hydrochloride (CPZ) was a gift of Smith Kline & French Laboratories of Philadelphia, PA. Doses are in mg salt per kg body weight. Control injections were pyrogen-free saline. T_b was measured at 30, 60, 90, 120, 180, 240, and 300 min postinjection.

Multiple pairwise comparisons were made by ANOVA followed by Scheffé's F-test. Values of t with a probability of less than 0.05 were considered statistically significant differences between means. All analyses were performed with the aid of Statview 512+ (Brain Power, Inc., Calabases, CA), and a Macintosh PC.

RESULTS

Preinjection control T_b readings in the guinea pig were remarkably stable, showing little intra- or interanimal variability; the baselines usually ranged from 39.0 to 39.3°C. There was little change in T_b over the 5-h testing period when the animals were injected with saline.

As illustrated in Fig. 1, dose-related changes in hypothermia were seen with the 3 doses of U. With 20 mg/kg the maximum drop in T_b was 2.9 ± 0.28°C; with 40 mg/kg, it was 4.6 ± 0.21°C, and with 80 mg/kg, it was 7.1 ± 0.69°C. After reaching a maximum at 90–120 min postinjection, the hypothermia gradually diminished but was still present when the experiment was terminated 5 h after the injections. For purposes of comparison, the effect of this drug in the rat [previously reported (1,4)] at the same doses is also shown. With the higher doses guinea pigs were relaxed but able to respond fully to stimuli. Fifty-seven percent of the animals with the two highest doses had periodic short bouts of shivering lasting for about 3 s, with the bouts recurring frequently, and 22% had splayed hind limbs for 30–180 min postinjection. Three out of the 8 animals receiving the highest dose of U died.

With CPZ, the magnitude of the changes was much less than that seen with U. The maximum decrease in T_b (irrespective of time postinjection) in a group given 2.5 mg/kg CPZ was 1.4 ± 0.14°C; it was 1.6 ± 0.24°C with the 5.0 mg/kg dose. These results were not statistically different from saline. The hypothermia peaked at 1 h postinjection and lasted about 2 h (Fig. 2). As would be expected with these doses of CPZ, the guinea pigs were sedated but were able to respond to stimuli.

Figure 3 illustrates the results with 3 different combinations of CPZ and U. When the two lower dose combinations of the drugs were used, there was a synergism of the hypothermic action that appeared to be greater than additive. This was noted especially with 2.5 mg/kg CPZ and 20 mg/kg U but was also seen to some extent with 2.5 mg/kg and 40 mg/kg U. Potentiation was manifested both in the peak effect and in the increase in duration of the marked hypothermia. When the highest doses of the two drugs (5 mg/kg CPZ and 80 mg/kg U) were administered, the maximum hypothermia was no greater than the effect seen with the kappa agonist alone. In addition, the peak effect occurred at about the same time as that with the individual drugs, and there appeared to be little difference in the duration of the effect. Fewer instances of shivering or splayed hind limbs were seen in animals given the combination than in animals given the U alone. Two of the 7 animals receiving the CPZ plus the 80 mg/kg dose of U died. Of the remaining 5, the data from 1 animal was not used because it was more than 2 S.D. from the mean.

Examination of the data in terms of the area under the time-response curve for the period of the experiment is shown in Table 1. The curves for 20 mg/kg U and the combination of that dose with 2.5 mg/kg CPZ were extrapolated to 300 min since measurements were only made for 240 min in those animals. Like the peak effect, the area under the curve shows a fairly stable response to CPZ, a dose-related increase in the hypothermia caused by U, and a potentiation of the effect of the individual drugs with the two lower dose combinations. [For U(20) vs. CPZ (2.5) + U(20): $F(8,42) = 3.092$; for U(40) vs. CPZ (2.5) + U(40): $F(8,42) = 3.395$, $p < 0.05$, ANOVA followed by Scheffé's.] Similarly, there was no synergism or potentiation of the effect of the individual drugs with the highest dose combination.

A rigorous statistical analysis to determine whether a true potentiation occurred would require many more dose combina-

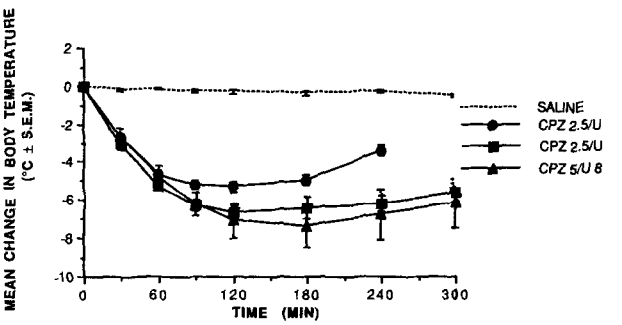

FIG. 3. Time-dependent effect of U50,488H (20, 40, or 80 mg/kg, SC) and chlorpromazine (2.5 or 5 mg/kg, SC) in combination on body temperature in guinea pigs. N=4–7 per group.

tions than we tested. However, in order to roughly assess whether the selected drug combinations had an effect greater than the effect of the individual drugs, we took the values at the peak effect of the drugs and compared the observed values with the calculated expected values (Dr. R. Tallarida, personal communication). The results are shown in Table 2. Thus the maximum average change in T_b occurred at 120 min for the combination of the lowest doses and was −5.3°C, whereas the calculated additive effect of the two drugs using values at the time of maximum change for each drug alone was −4.3°C. For the combination of 2.5 mg/kg CPZ and 40 mg/kg U, the peak effect was −6.7°C at 120 min postinjection. The additive effect of the two drugs would have been −6.0°C. Note that the two lowest dose combinations had an observed effect greater than that of the calculated value for the combination of the individual effects. The observed effect with the highest dose combination was less than that of the calculated value.

DISCUSSION

We stated in the introduction that we began this study with two postulates: 1) the dose-response curve for the hypothermia produced by a selective kappa agonist alone would be shifted to the left in the guinea pig when compared to the rat; 2) a potentiation would result when chlorpromazine and the kappa agonist were used simultaneously. The first postulate was clearly proven, whereas the second is supported to only a limited extent.

In terms of the effect of U alone, the peak effect in the rat with the maximum dose used here (80 mg/kg) was a drop in T_b of approximately 2°C (1,4). In contrast, the hypothermia produced by the dose in the guinea pig was 7.1°C. Even a dose of 160 mg/kg in the rat resulted in a drop of T_b of only 4°C. Similarly, the lowest dose tested in the guinea pig (20 mg/kg) produced a drop of 2.9°C, whereas the drop in the rat with that dose was 0.5°C. In addition, the duration of the hypothermia produced in the guinea pig was much greater than that seen in the rat.

TABLE 1

AREA UNDER THE CURVE (0–300 MIN) FOR THE HYPOTHERMIC EFFECT

Injections (dose in mg/kg)	N	Mean Area
Saline/Saline	5	−82.20
CPZ (2.5)/Saline	6	−225.75
CPZ (5)/Saline	6	−276.00
U (20)/Saline	6	−551.15
U (40)/Saline	7	−978.53
U (80)/Saline	5	−1687.50
CPZ (2.5)/U (20)	7	−1170.96*
CPZ (2.5)/U (40)	5	−1662.15*
CPZ (5)/U (80)	4	−1759.69

*Significantly greater than U alone, $p<0.05$, ANOVA followed by Scheffé's F-test.

If we analyze the data in terms of the area under the time-response curve for the 5 h of the experiment (Table 1), the results with U are also dose-related. Compared to the rat (4), the effect is markedly greater in the guinea pig. For the 20 mg/kg dose, the area is 523% higher in the guinea pig. For 40 mg/kg, it is 374% higher and for 80 mg/kg, it is 296% higher.

Thus whether we analyze the data on the basis of peak effect or on the basis of area under the time-response curve, it is clear that the kappa agonist has a greater effect in the guinea pig than in the rat. Since the guinea pig has been shown to have a much greater percentage of kappa receptors in the central nervous system than the rat (14), and since we have postulated that the kappa receptors are responsible for hypothermic actions in thermoregulatory control mechanisms, the findings in this experiment lend further support for our hypothesis. Although there may be more than one subtype of kappa receptor present in the guinea pig brain, U has been reported to act on two of those subtypes (6, 15, 16). As mentioned above, an earlier study with relatively nonselective opioids and opioid peptides given by the ICV route found predominantly hyperthermic responses in guinea

TABLE 2

OBSERVED VS. EXPECTED VALUES FOR CHANGE IN BODY TEMPERATURE (°C)

		$\text{Mean}_{CPZ} + \text{Mean}_{U} \pm \sqrt{(\text{S.E.M.})^2_{CPZ} + (\text{S.E.M.})^2_{U}}$	
		CPZ + U (expected)	CPZ + U (observed)
CPZ (2.5 mg/kg)	U (20 mg/kg)		
−1.4 ± 0.14	−2.9 ± 0.28	−4.3 ± 0.31	−5.3 ± 0.29
CPZ (2.5 mg/kg)	U (40 mg/kg)		
−1.4 ± 0.14	−4.6 ± 0.21	−6.0 ± 0.25	−6.7 ± 0.41
CPZ (5.0 mg/kg)	U (80 mg/kg)		
−1.6 ± 0.24	−7.1 ± 0.69	−8.7 ± 0.73	−7.4 ± 1.07

pigs (12). In that study, doses of morphine, ketazocine, SKF 10047, or pentazocine greater than 100 μg caused hypothermia. All of these drugs have some kappa activity, but none, including the prototype kappa agonist ketazocine, is now considered to be selective for that receptor. In rats, ethylketazocine, SKF 10047, and pentazocine caused hyperthermia even in doses above 100 μg ICV (10), possibly indicating a lesser sensitivity to kappa effects as a result of the presence of greater numbers of mu receptors. Morphine could not be tested at such high doses because of toxicity but caused hyperthermia in doses up to 65 μg ICV. By SC injection, ethylketazocine and ketazocine produced only hypothermia, while morphine elicited hyperthermia at lower doses and hypothermia with higher ones (8). Qualitative differences in temperature effects as a result of route of administration are not uncommon (2,5) and could be explained by differences in receptor distribution and ability of the drug to reach its sites of action. One other point bearing on drug sensitivity should also be mentioned. In the guinea pig studies, 37% of the subjects receiving the highest dose of U died and 29% receiving this dose in combination with 5 mg/kg CPZ died. In our rat studies, there were no deaths at 20°C ambient even with doses of U as high as 160 mg/kg.

With regard to our postulate that we would see a potentiation when CPZ was coadministered with U, the results shown in Table 1 demonstrate that there is a greater than additive effect at the two lower dose drug combinations, with the effect being 38–50% more than the effect of these drugs individually. In the rat, the comparable percentage change is 185–239% (4). With the highest dose combination used in the guinea pig (2.5 mg/kg CPZ and 80 mg/kg U), the effect is 10% less than the added effects of the individual drugs, whereas in the rat this dose combination is 104% greater than additive. Thus, although the prediction of a potentiation holds up for the lower dose combinations, it does not for the highest dose combination. Furthermore, at all dose combinations, the potentiated effect is far greater in the rat than in the guinea pig. Although we cannot fully explain the differences, there are a number of possibilities that could be considered. One is that we have reached the maximum hypothermic effect that can be seen in the guinea pig with these pharmacologic agents. It is possible that the response to the drug combination in the guinea pig is more of a regulated response than in the rat. The shivering seen in the guinea pigs with the highest dose of U, at which there was the greatest drop in T_b, is likely due to an attempted thermoregulatory response and indicates that a poikilothermia is not produced by the drug. Although no shivering was noted in the rat, preliminary findings (unpublished) in our laboratory indicate at least a minimal attempt at thermoregulation. One would also have to consider the possibility that the interaction between CPZ and U is different in the guinea pig than in the rat. Whether this might be due to differences in the dopamine or other neurotransmitter systems related to thermoregulation in the guinea pig is not known at the present time. It may be of interest to point out, however, that the relationship between behavioral and autonomic thermoregulation in the guinea pig is considerably different from that observed in other rodent species such as the mouse and hamster (11). It is tempting to speculate that kappa opioid receptors might be involved in these differences.

In conclusion, we have demonstrated that the hypothermic action of the selective kappa agonist U is far greater in the guinea pig than in the rat. Furthermore, the combination of the kappa agonist and CPZ produces a greater than additive effect when used in low doses in the guinea pig, but the degree of potentiation is much less in the guinea pig than in the rat.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Ronald Tallarida for his help in analyzing the data, and Dr. John McCall and Mr. Irwin Greenberg of The Upjohn Co. for their assistance in obtaining U50,488H. This study was supported in part by grant DA 00376 from the National Institute on Drug Abuse.

REFERENCES

1. Adler, M. W.; Geller, E. B. Hypothermia and poikilothermia induced by a κ-agonist opioid and a neuroleptic. Eur. J. Pharmacol. 140:233–237; 1987.
2. Adler, M. W.; Geller, E. B. Physiological functions of opioids: Temperature regulation. In: Herz, A.; Akil, H.; Simon, E. J., eds. Opioids. Berlin: Springer-Verlag; in press.
3. Adler, M. W.; Geller, E. B.; Rosow, C. E.; Cochin, J. The opioid system and temperature regulation. Annu. Rev. Pharmacol. Toxicol. 28:429–449; 1988.
4. Adler, M. W.; Geller, E. B.; Rowan, C. H.; Pressman, N. Profound reversible hypothermia induced by the interaction of a kappa-agonist opioid and chlorpromazine. In: Cooper, K.; Lomax, P.; Schönbaum, E.; Veale, W. L., eds. Homeostasis and thermal stress. Basel: Karger; 1986:160–162.
5. Burks, T. F. Opioids and opioid receptors in thermoregulation. In: Schönbaum, E.; Lomax, P., eds. Thermoregulation: Pathology, pharmacology and therapy. New York: Pergamon; 1991:489–508.
6. Clark, J. A.; Liu, L.; Price, M.; Hersh, B.; Edelson, M.; Pasternak, G. W. Kappa opiate receptor multiplicity: Evidence for two U50,488-sensitive κ_1 subtypes and a novel κ_3 subtype. J. Pharmacol. Exp. Ther. 251:461–468; 1989.
7. Clark, W. G.; Lipton, J. M. Changes in body temperature after administration of amino acids, peptides, dopamine, neuroleptics and related agents: II. Neurosci. Biobehav. Rev. 9:299–371; 1985.
8. Geller, E. B.; Hawk, C.; Keinath, S. H.; Tallarida, R. J.; Adler, M. W. Subclasses of opioids based on body temperature change in rats: Acute subcutaneous administration. J. Pharmacol. Exp. Ther. 225:391–398; 1983.
9. Geller, E. B.; Hawk, C.; Tallarida, R. J.; Adler, M. W. Postulated thermoregulatory roles in different opiate receptors in rats. Life Sci. 31:2241–2244; 1982.
10. Geller, E. B.; Rowan, C. H.; Adler, M. W. Body temperature effects of opioids in rats: Intracerebroventricular administration. Pharmacol. Biochem. Behav. 24:1761–1765; 1986.
11. Gordon, C. J. Relationship between behavioral and autonomic thermoregulation in the guinea pig. Physiol. Behav. 38:827–831; 1986.
12. Kandasamy, S. B.; Williams, B. A. Hyperthermic responses to central injections of some peptide and nonpeptide opioids in the guinea-pig. Neuropharmacology 22:621–628; 1983.
13. Laborit, H.; Huguenard, P. L'hibernation artificielle par moyens pharmacodynamiques et physiques. Presse Med. 59:1329; 1951.
14. Robson, L. E.; Gillan, M. G. C.; Kosterlitz, H. W. Species differences in the concentrations and distributions of opioid binding sites. Eur. J. Pharmacol. 112:65–71; 1985.
15. Tiberi, M.; Magnan, J. Pharmacological characterization of the binding of [^{3}H]-bremazocine in guinea-pig brain: Evidence for multiplicity of the κ-opioid receptors. Can. J. Physiol. Pharmacol. 67: 1336–1344; 1989.
16. Zukin, R. S.; Eghbali, M.; Olive, D.; Unterwald, E. M.; Tempel, A. Characterization and visualization of rat and guinea pig brain κ opioid receptors: Evidence for κ_1 and κ_2 opioid receptors. Proc. Natl. Acad. Sci. USA 85:4061–4065; 1988.