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TRIMU-5, a μ_2-Opioid Receptor Agonist, Stimulates the Hypothalamo–Pituitary–Adrenal Axis

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EISENBERG, R. M. *TRIMU-5, a μ_2-opioid receptor agonist, stimulates the hypothalamo-pituitary-adrenal axis.* PHARMACOL BIOCHEM BEHAV 47(4) 943–946, 1994.—Previous work in our laboratory has shown that DAMGO (ICV) will cause an elevation in plasma corticosterone (CS). The effect was blocked by pretreatment with β-FNA but not by naloxonazine, suggesting indirectly that DAMGO's effect was via a μ_2-opioid receptor. TRIMU-5, a μ_2 agonist/μ_1 antagonist, was tested in a similar series of experiments to show more directly that the effect of DAMGO to increase plasma CS was via the μ_2 receptor. Experiments were conducted on conscious, unrestrained, male Sprague–Dawley rats with chronic IV catheters and ICV cannula guides allowing for serial blood sampling and drug injection into the right lateral ventricle. During this process, animals remained isolated in sound-attenuated one-way vision boxes. TRIMU-5, 50 μg, produced a sustained increase in plasma CS for a 3-h period. The response peaked at 30 min, showing a plasma CS level of 19.7 ± 1.4 μg/dl. A lower dose, 10 μg, did not produce a significant response. A higher dose, 100 μg, produced an elevated hormone response in a pilot study but was lethal in half the animals. The plasma CS increase was blocked by pretreatment with β-FNA, 20 μg ICV, given 18 h before TRIMU-5, but was unaffected by naloxonazine pretreatment, 20 mg/kg IV, also administered 18 h before TRIMU-5. These data confirm our earlier conclusion that the effect of DAMGO to elevate plasma CS was through a μ_2-opioid receptor.

TRIMU-5 μ_2-Opioid receptor Corticosterone

THE action of morphine on many processes occurs by an interaction with a variety of receptors so that the result may be considered a composite of these interactions. Such is probably the case for the increase in plasma adrenal glucocorticoid levels following the acute administration of morphine (1,2, 7,22,24). This activation of the hypothalamo–pituitary–adrenal (HPA) axis results from the stimulation of μ, κ, and δ receptors, as well as σ and ϵ receptors, producing a facilitatory effect. The differentiation of the opioid receptor into the current spectrum of types and subtypes has been made possible by the development of ligands, with selective agonist and antagonist properties, and by the refined binding techniques to demonstrate these affinities. In addition to the prototypic μ agonist morphine, DAMGO (12,14) as well as a number of other more or less μ-selective peptides (2,18,24) have been reported to increase plasma corticosterone (CS). Agents directed at the κ group of receptors have also been shown to stimulate the HPA axis (2,3,7,16–19,21,22,24). DPDPE, a selective δ agonist, elevated plasma CS levels [(12), Eisenberg, unpublished observations]. The possibility of inhibitory opioid influence has been suggested by reports that both naloxone and naltrexone, particularly at high doses, will increase plasma CS levels by a central mechanism (5,6,9).

Recent work in our laboratory has examined the action of DAMGO (4). The findings suggest that the stimulation of the HPA axis following DAMGO results from an activation of μ_2 and not μ_1 receptors. Plasma CS elevation following the ICV administration of this peptide was blocked by β-FNA but not naloxonazine. β-FNA is selective for both μ receptor subtypes, whereas naloxonazine was μ_1 selective at the times TRIMU-5 was tested. Nor-BNI and naltrindole are κ- and δ-selective antagonists, respectively. Naloxonazine was ineffective in blocking the corticosterone response; however, it was effective in blocking the antinociception produced by DAMGO in the same animals. Further, DAMGO's effect was opioid selective, as neither β-FNA nor naloxonazine pretreatment altered the characteristic HPA response to ether vapor exposure.

It is only by inference that the effect of DAMGO is suggested as μ_2 mediated. In fact, the evidence only supports that it is not μ_1 mediated. In the present study, we use a new compound, TRIMU-5, to provide a more direct demonstration that the action of DAMGO *is* μ_2 mediated. TRIMU-5 is

an enkephalin analog with high affinity for both of the μ sites, with poor affinity for other opioid receptors. It was capable of producing analgesia in the mouse, which was blocked by β-FNA but not naloxonazine. Binding studies have characterized this agent as a μ_2 agonist and a μ_1 antagonist (25). Thus, this agent appears to possess unique properties by which to examine the μ-opioid receptor activation of the HPA axis.

METHOD

Animals

Male Sprague-Dawley-derived rats (SASCO, Madison, WI) weighing 200–350 g were utilized in these experiments. These have been found to be of quiet temperament and routinely show basal plasma CS levels of 6–10 μg/dl. Rats were housed in pairs until surgery and then were maintained individually until the day of the experiment. This was found to be necessary to prevent tampering with the acrylic appliance by cage mates. They were maintained at constant temperature (21 °C) and light cycle (lights on at 0600 to 2000 h). Standard laboratory chow and water were provided ad lib to all animals. Each animal was allowed to acclimate to the surroundings and routine for at least 5 days prior to beginning the experiment.

Surgical and Experimental Procedure

The experimental procedure involved three phases: 1) surgical preparation; 2) a 3- or 4-day recovery period during which animals were acclimatized to the experimental environment; and 3) the experiment during which animals were placed into inverted hanging mouse cages placed within the isolation chambers, test drugs were injected, and serial blood samples were withdrawn for hormone determinations. For the surgical placement of the IV catheter, each animal was anesthetized (pentobarbital, 50 mg/kg, IP) and gentamicin sulfate (5 mg/kg, IP) was injected prior to incision. The tip of the silicone rubber catheter (Silastic®) was inserted via the external jugular vein to the entrance of the right atrium as already described (8). The ICV injection guides were constructed as previously described (4). Each guide was placed 0.2 mm posterior to bregma and 1.6 mm right lateral to midline and to such a depth so the tip of the plug or injection cannula entered the lateral ventricle according to the atlas of Pellegrino and Cushman (23). To maintain catheter patency during the interval between surgery and blood sampling, catheters were flushed with 0.1–0.2 ml heparinized isotonic saline, 500 U/ml. In this 3–4-day recovery/acclimatization period between surgery and experimentation, the animals were placed into the experimental chambers so that the stress of a novel environment would not be a factor. On the day of the experiment, animals were introduced into individual sound-attenuated chambers and connected to the externalized injection/sampling tube. The ICV cannula (preloaded with drug) was inserted into the guide so the tip entered the lateral ventricle. This system allowed for ICV drug injection and serial blood sampling from conscious, unrestrained animals. The animals were connected at 0630 h. At least 2 h were allowed to elapse after the animal's placement and connection so the plasma CS could return to basal level after handling. Blood sampling into heparinized syringes began at 0900 h. The treatment drug was injected after the zero-time sample. Additional samples were obtained at 15, 30, 60, 120, and 180 min following drug injection. Each 0.6-ml blood sample was withdrawn following the removal of the void volume of the sampling tube. To reduce the effects of blood loss involved with sequential sampling, the fluid volume was replaced by isotonic saline after the first sample. After each subsequent sampling of blood, the cellular fraction from the previous sample, suspended in isotonic saline, was injected. At the end of each series of blood sampling, samples were transferred to test tubes and centrifuged, and the plasma separated and stored at 0°C. Plasma CS was determined by a modification of the fluorometric method of Glick et al. (10). In this procedure, methylene chloride was substituted for chloroform in the extraction of CS and the dilute sodium hydroxide was omitted. The assay has a working range of 1–60 μg/dl, an intra-assay variability of $< 2\%$, and an interassay variability of $< 4\%$. At the conclusion of the experiment, the animals were anesthetized and 5 μl of India ink was injected using a similar injection cannula. The animals were decapitated; the brains were removed and grossly dissected. The presence of ink in the third ventricle and the infundibular stalk was used as the criterion for a proper injection and inclusion in the data pool.

Drugs

Drugs administered ICV were in a volume of 5 μl and injected over approximately 15 s. When drugs were to be injected after the animal was placed into the isolation chamber, the injection cannula was preloaded and the cannula remained in place for the duration of the session. When injections were made on the afternoon prior to experiment, the cannula remained in the guide for 15 s after the injection to prevent any back flow. β-FNA and naloxonazine were purchased from Research Biochemicals, Inc. (Natick, MA). TRIMU-5 was generously provided by Dr. Bernard Roques (UFR des Sciences Pharmaceutiques et Biologiques, Paris, France). Solvents for each of the drugs, which also served as control vehicles, are the following: TRIMU-5/isotonic saline, β-FNA/distilled water, and naloxonazine/saline : 0.1 N acetic acid (1 : 1). Naloxonazine for IV administration was made up so that the fluid volume was 1.0 ml/kg b.wt. Other drugs were administered ICV at times indicated in the Results section below.

Statistics

In analyzing the data, a repeated measures ANOVA was used to compare all posttreatment values at all time intervals

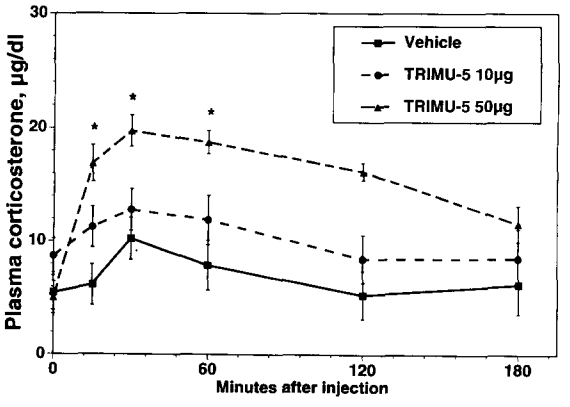

FIG. 1. The effects of TRIMU-5 on plasma corticosterone. The enkephalin analog was administered ICV after the zero-time blood sample. Error bars show mean ± SEM, $*p < 0.05$ (6–7 animals/group).

with control values. Where there was a significant difference in the posttreatment response curves, post hoc analysis of the individual time points was done using factorial ANOVA. Levels of significance are indicated with $p < 0.05$ using Bonferroni's t-test for multiple group comparisons.

RESULTS

The results in Fig. 1 show the effects of two doses of TRIMU-5 on plasma CS. The higher of the two doses (50 μg/5 μl, ICV) produced a significant increase in hormone level for at least 60 min whereas the lower dose had no effect. A higher dose, 100 μg, also stimulated the HPA axis in a pilot study (results not shown) but was lethal to approximately half of the animals tested. Thus, TRIMU-5 appears to have a rather narrow working range in this system in the rat, even though the effective dose was the same as that used to produce antinociception in mice (25).

Pretreatment with β-FNA, 20 μg ICV, given at 18 h before, effectively blocked the stimulation of the HPA axis by TRIMU-5 (Fig. 2). In contrast, similar pretreatment with naloxonazine, 20 mg/kg IV, had no effect on the response to TRIMU-5 (Fig. 3).

DISCUSSION

Previous work (4) determined that DAMGO elevated plasma CS by a mechanism that was not μ_1 mediated. The effect was through the μ-opioid receptor because it could be blocked by pretreatment with β-FNA but not with naloxonazine (a μ_1-selective antagonist). DAMGO is considered to be a highly selective and potent μ agonist according to the "ligand selectivity profile" developed by Goldstein and Naidu (11). Similar responses to these doses have been observed by others (12,14). Gunion et al. (14) also observed a reduction of the agonist effect by pretreatment with β-FNA (20 μg, ICV). The development of the enkephalin analog, TRIMU-5, a selective μ_2-selective agonist, allows for the next piece of evidence showing that DAMGO's effect is μ_2 mediated (25).

TRIMU-5 shows high binding affinity for both of the μ sites, with poor affinity for other opioid receptors. In the mouse, it produced analgesia that was blocked by β-FNA,

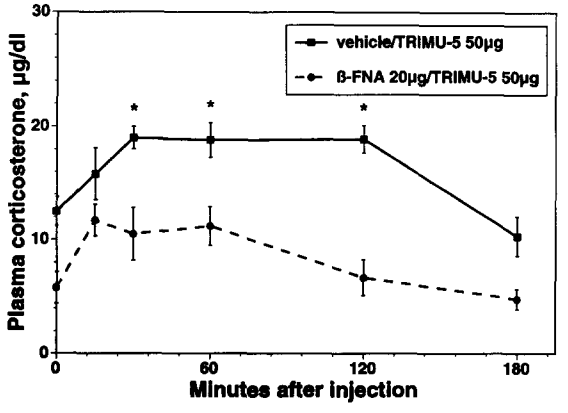

FIG. 2. The effects of TRIMU-5 on plasma corticosterone 18 h following the ICV administration of β-FNA. The enkephalin analog was administered ICV after the zero-time blood sample. Error bars show mean ± SEM, $^*p < 0.05$ (6 and 7 animals/group, respectively).

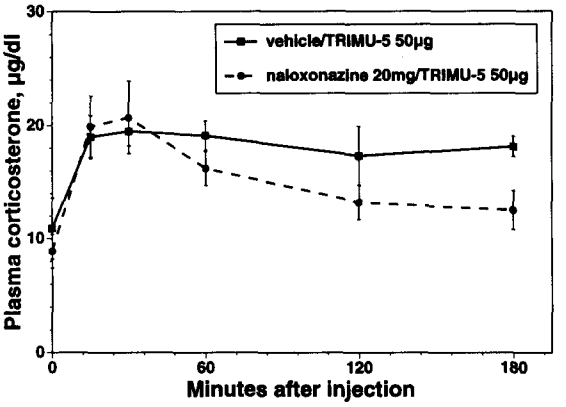

FIG. 3. The effects of TRIMU-5 on plasma corticosterone 18 h following the IV administration of naloxonazine, 20 mg/kg. The enkephalin analog was administered ICV after the zero-time blood sample. Error bars show mean ± SEM, $^*p < 0.05$ (5 and 11 animals/group, respectively).

an irreversible μ antagonist (26), but not naloxonazine, a μ_1-selective antagonist (15). Specifically, binding studies have characterized TRIMU-5 as a μ_2 agonist and a μ_1 antagonist. In the analgesia studies, the ICV dose required was approximately two orders of magnitude greater than that required by IT administration. This might suggest that the number of μ_2 receptors accessible by the ICV route may be substantially less by other routes. The activation of the HPA axis also required a similar dose and exhibited a narrow range, in keeping with our previous work with DAMGO. This is substantially higher than doses required to produce other responses, analgesia for example.

The high doses and the possible transport via CSF to a number of areas suggest that TRIMU-5, like DAMGO, may act by both direct and indirect means to elevate plasma CS. Because the hormone response to TRIMU-5 occurs by the first measurement at 15 min, the trigger must be rapid. Direct stimulation of pathways causing the release of corticotropin-releasing factor is one possibility—so might be indirect respiratory depression/hypoxia or pressor effects that result in a stress response. More exaggerated effects with higher doses of TRIMU-5 may explain the lethality that was observed in pilot experiments. Other possible effects such as disruption of electrolyte balance or acid/base balance probably do not occur rapidly enough to explain the immediate rise in hormone level. Those may, however, contribute to sustaining the response. Whether it is an indirect trigger, or some point in the final common pathway for corticotropin-releasing factor secretion that is stimulated, it appears to be μ_2 receptor specific, as the response was blocked by β-FNA but was unaffected by naloxonazine. Even though this activation of the HPA axis has opioid receptor involvement, endogenous opioids do not appear to participate in maintaining basal plasma CS. Zero-time plasma CS levels were not altered in animals pretreated with either β-FNA or naloxonazine.

The effects of TRIMU-5 and β-FNA suggest that a sufficient number of μ_2 receptors mediating the HPA activation are accessible via the ventricular CSF. Moskowitz and Goodman (20) observed that binding to μ_2 receptors was moderate to high in the hypothalamus and limbic system of the mouse. Findings of similar μ_2 binding in the rat have also been made (13).

The evidence that DAMGO acts via μ_2 receptors to activate the HPA axis is becoming stronger. Our initial findings showed that the response to DAMGO was not altered by the μ_1-selective antagonist naloxonazine but was blocked by β-FNA. The current findings show that a μ_2-selective agonist stimulates the HPA axis and this was blocked by β-FNA but not by naloxonazine. Perhaps the final piece of evidence will come with the development of a selective μ_2 antagonist.

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