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Limitations to Effect of α-MSH on Permeability of Blood-Brain Barrier to IV ^{99m}Tc-Pertechnetate

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KASTIN, A. J. AND L. A. FABRE. *Limitations to effect of α-MSH on permeability of blood-brain barrier to IV ^{99m}Tc-pertechnetate.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1199–1201, 1982.—The effects of several variables on the permeability of the blood-brain barrier (BBB) to ^{99m}Tc-labeled sodium pertechnetate after IV administration of α-MSH were investigated. Doses of α-MSH of about 200 μg/kg were generally more effective in increasing the brain:blood ratio of radioactivity than the smaller doses that had previously been shown to affect behavior and the EEG. Pulsatile administration of a total of 200 μg/kg α-MSH over 90 min did not change the permeability of the BBB to the pertechnetate anion. Infusion of the same dose over 90 min significantly increased the brain:blood ratio of radioactivity in one of two experiments: no significant effects were seen with infusion for shorter times, lower concentrations, or with a 4–9 analog (Org 2766). In another experiment, bolus injection of 200 μg/kg α-MSH resulted in a significantly increased ratio 90 min later as compared with controls. Although the effects of a peptide on the permeability of the BBB to other compounds remains intriguing, limitations appear to exist in experiments with ^{99m}Tc-pertechnetate.

Blood-brain barrier α-MSH Peptide CNS Anion

THERE are several ways by which peripherally injected peptides like α-melanocyte-stimulating hormone (α-MSH) can exert their effects on the central nervous system (CNS) [6]. Most of the work in this controversial area has investigated the passage of small amounts of peptides across the blood-brain barrier (BBB) in intact form [1,5].

Another of the possibilities involves alteration in the permeability of the BBB to other substances after peripheral administration of a peptide. The previous attempt to investigate this effect on the BBB showed that intravenous (IV) α-MSH increased the ratio in the brain:blood of the small inorganic anion pertechnetate to a small but statistically significant extent [14]. The present study further examined this finding.

METHOD

The method used was essentially the same as that previously described [2,14] except that a larger volume of blood was counted. Male, albino rats obtained from Blue Spruce Farms (Altamont, NY) and weighing about 250 g were injected IV with sodium pertechnetate labeled with ^{99m}Tc. This was immediately followed by the IV administration of peptide or diluent (0.9% NaCl made to 0.01 M with acetic acid) rapidly as a bolus, as a continuous infusion through a Harvard Apparatus Compact Diffusion Pump (South Natick, MA), or as repeated injections of a bolus every 10 min. At various times after the ^{99m}Tc-pertechnetate tracer, the thoracic cavity was opened, about 0.5 ml of blood was obtained from the right ventricle, and 120 ml of 0.9% NaCl was perfused through the left ventricle to clear the cerebral vasculature of the radioactive tracer. The brain (minus pituitary, pineal, and hypothalamus) was removed, rinsed, and counted as was the sample of blood. The results were expressed as the ratio of radioactivity in the brain to that in the blood × 100. Statistical significance was determined by analysis of variance followed by Duncan's Multiple Range Test.

RESULTS

Experiment 1: Time after Injection of 100 μg/kg α-MSH as a Bolus

The effects of a single rapid injection of a bolus of 100 μg/kg α-MSH were examined 0, 1, 5, 10, 15, 30, 45, 60, 90, and 120 min after injection of ^{99m}-Tc-labeled sodium pertechnetate and the peptide. Analysis of variance revealed a significant main effect of time, $F(9,100)=2.21$, $p<0.05$, but not of treatment; the interaction was also not significant. The highest ratios of counts (brain:blood) occurred with α-MSH at 60 and 90 min, but the ratios at these times after injection of the diluent were also higher than at time 0.

Experiment 2: Doses of α-MSH from Previous Study Injected as a Bolus

Although all the experiments in this study were being performed by a different individual than in the previously published study [14], it was considered possible that the slightly different dose used in Experiment 1 (100 μg/kg) as compared with the previous study (80 and 160 μg/kg) might

have contributed to the lack of a statistically significant effect of treatment. Accordingly, in Experiment 2 we used α-MSH in doses of 80 and 160 μg/kg as well as a dose of 40 μg/kg, doses found effective in previous behavioral studies [8]. Based on the results of Experiment 1 and our desire to check a longer time interval than that used in the previous study [1,4], all rats were killed at 90 min.

Analysis of variance showed a significant main effect for dose, $F(3,10)=3.72$, $p<0.05$. By Duncan's Multiple Range Test, only the 160 μg/kg dose of α-MSH resulted in a significantly higher ratio (4.5) than that caused by injection of the diluent (3.1). The effect of the 160 μg/kg dose was also significantly ($p<0.05$) higher than that of the 40 and 80 μg/kg doses of α-MSH which did not differ reliably from the control.

Experiment 3: Comparison of Different Methods of IV Administration of 200 μg/kg α-MSH

Experiment 2 showed that a higher dose of the peptide caused a greater effect than lower doses. For convenience, we selected the dose of 200 μg/kg α-MSH and compared its effect to that of diluent at 90 min by different methods of injection. The same dose of peptide was administered by constant infusion over 90 min (at a rate of 2.2 μg/kg-min) or in 9 divided doses given as pulses as well as by injection as a single bolus. A total of 24 rats were used, each group containing 4 animals.

Although separate groups of rats receiving diluent were used as controls for each of the 3 methods of administration, their mean ratios (3.8) were identical. Rats given 200 μg/kg α-MSH as a single bolus and killed 90 min later had a ratio of 4.6. Division of the same dose of α-MSH into 9 equal parts given once every 10 min resulted in a ratio of 4.0. Infusion of 200 μg/kg over 90 min resulted in a ratio of 6.6, an increase over controls of 2.8. The significant main effect of treatment, $F(1,18)=5.93$, $p<0.05$, was explained by the significant ($p<0.05$) difference of the α-MSH given by infusion for 90 min as compared with all the other 5 groups (α-MSH by bolus or pulse) and the 3 diluent groups.

Experiment 4: Infusion of α-MSH for Shorter Times

Since the onset of the changes in the EEG after IV injection of α-MSH occur sooner than 90 min [10,13], we repeated the infusion rate of 200 μg/kg over 90 min (2.2 μg/kg-min) for shorter time periods. These resulted in infusion of total doses of about 11 μg/kg at 5 min, 33 μg/kg at 15 min, 66 μg/kg at 30 min, and 133 μg/kg at 60 min. Separate groups of rats were infused with a total of 200 μg/kg α-MSH at each of these same times. In each group, 2–5 rats were used.

The differences in the ratios of the rats receiving a total of 200 μg/kg α-MSH by infusion at each time were slightly higher than the appropriate controls at 15 min (1.2), 30 min (0.9), and 60 min (0.4). Neither these differences, however, nor any of those involving the lower doses were statistically significant.

Experiment 5: Infusion of an Analog and Fragment of α-MSH

A 4–9 analog of MSH/ACTH, H-Met(0)-Glu-His-Phe-D-Lys-Phe-OH, made by Organon (Org 2766) has been reported to be behaviorally 1000 times more potent than α-MSH or the 4–10 fragment of MSH/ACTH [3]. It was possible that this analog might also be more active on the permeability of the BBB to labeled pertechnetate. These 3 peptides were tested at the same dose (200 μg/kg) and route of administration (infusion) used in Experiments 3 and 4, and killed at 90 min. In addition, the tri-substituted MSH/ACTH 4–9 analog was tested at a dose 1000 times smaller (0.2 μg/kg).

The ratios of radioactivity in the brain to that in the blood were found to be similar for all five groups (diluent, α-MSH, MSH/ACTH 4–10, and Org 2766 at two doses). The range was narrow (3.5–3.9), and none of the treatments differed to a reliable extent.

Experiment 6: Injection of 200 μg/kg α-MSH as a Bolus

The inconsistency of the results after infusion of 200 μg/kg α-MSH led to general doubts about the efficacy of the peptide on the permeability of the BBB to ^{99m}Tc-labeled sodium pertechnetate. Accordingly, the mode of administration of α-MSH originally found effective was again used.

Ten rats received 200 μg/kg α-MSH and 10 rats the diluent as a bolus injection. The difference in the brain:blood ratio of the rats injected rapidly with α-MSH was higher (1.0) than the ratio in the rats injected similarly with the diluent. This difference was statistically significant, $F(1,18)=7.07$, $p<0.05$.

DISCUSSION

The results show that the effect of α-MSH in changing the permeability of the BBB to ^{99m}Tc-pertechnetate is sensitive to a number of variables. One of these was shown to be dose. When injected rapidly as a bolus, significantly higher brain:blood ratios of radioactivity were found with doses of 160 μg/kg and 200 μg/kg (Experiments 2 and 6), but not with smaller doses (Experiments 1 and 2). This indicates that the inverted U-shaped dose-response curve we have been finding for a number of years [6] was not evident at this range. In an experiment (Experiment 3) designed to test another variable, however, the higher ratio found in the 4 rats receiving 200 μg/kg of α-MSH was not statistically different from that of the 4 rats receiving diluent.

The variable tested in Experiment 3 was mode of administration. The ratio after infusion of 200 μg/kg α-MSH was significantly higher than that after injection of α-MSH as a bolus or in multiple pulses. This raised the possibility that CNS effects of α-MSH [3,7] could be enhanced by longer exposure to the peptide. It might have supported the use by DeWied's group as recently as 1977 [15] of the zinc phosphate solution designed to prolong the activity of α-MSH administered peripherally. By itself, however, this vehicle can cause behavioral effects similar to those of α-MSH [8] and is not necessary for the actions of α-MSH after peripheral administration [7]. Since peptide analogs have been developed with longer half-lives than their natural counterparts, the results from Experiment 3 involving infusion also raised the possibility that, in addition to their preferential structural characteristics, the prolonged life of analogs might alter their efficacy at the BBB. The use of α-MSH in an experiment (Experiment 5) designed to compare its effects by infusion with that of two related compounds, however, failed to show a reliable difference, even though the 4–9 analog (Org 2766) has been reported to have greatly increased potency in some [3] but not all [12] experimental situations.

Another variable that seemed to affect the results was time. Although the half-time disappearance of α-MSH is short [4, 9, 11], its effects on behavior [7] and the EEG

[10,13] have been shown to start in about 15 min and persist much longer. The lack of effect of α-MSH when infused for periods up to an hour (Experiment 4) seemed to reflect a different time variable. Subsequent doubts generated by Experiment 5 about any greater efficacy of this method of administration makes it difficult to interpret the negative results of Experiment 4 involving time.

The most consistent effect seemed to be found after injection of 200 μg/kg α-MSH as a bolus at 90 min. Since most of the behavioral and EEG actions of α-MSH and its related compounds have been found at smaller doses and shorter times after injection, the meaning of the effects of α-MSH on the BBB are not clear. The use of the unphysiologic anion ^{99m}Tc-pertechnetate that may be actively removed from the brain emphasizes another of the difficulties in interpretation of the results. Despite limitations to the effects of α-MSH in altering the permeability of the BBB, the results do not exclude the possibility that more than one mechanism may be involved in the actions of peripherally administered peptides on the brain.

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