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Neuropeptides and the Blood-Brain Barrier in Goldfish[1]

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OLSON, R. D., A. J. KASTIN, D. MONTALBANO-SMITH, G. A. OLSON, D. H. COY AND G. F. MICHELL. *Neuropeptides and the blood-brain barrier in goldfish.* PHARMAC. BIOCHEM. BEHAV. 9(4) 521–524, 1978.—The general activity level of a goldfish is easily monitored by placing it in water to a depth of 2.5 cm in an aquarium on top of an activity meter. With this system, goldfish were administered a 5 μl (80 μg/kg) intracranial (IC) or intraperitoneal (IP) injection of one of 21 compounds and tested for general activity. The results indicated that activity decreased significantly over time and that the peptides differentially decreased activity, with the greatest alterations in activity produced by two new enkephalin analogs: D-Ala^2, F$_5$Phe^4-enkephalin-NH$_2$ and N$^{\alpha}$N$^{\epsilon}$-bis(D-Ala^2-enkephalin)-Lys-NH$_2$. Overall, decreased activity began approximately 3 min after an IC injection and 6 min after an IP injection. The longer latency after IP injections may indicate the time required for the substance, either in its original or fragmented form, to reach and cross the blood-brain barrier and makes a primary peripheral effect unlikely. Most of the peptides or possibly their metabolites appeared to enter the brain since no significant difference in activity existed after IC and IP injections, with both producing reliable decreases from the control. In summary, peptides can exert behavioral effects after both IC and IP administration in goldfish.

MIF-1 α-MSH Substance P Neurotensin Somatostatin DSIP Enkephalin Endorphin
Blood-brain barrier Activity Goldfish

ALTHOUGH central injections of many neuropeptides produce significant behavioral effects [3, 12, 15, 23], it has not been firmly established at this time whether the opiate and some other peptides are able to cross the blood-brain barrier and produce comparable effects after peripheral injection. Kastin *et al.* [18] found that IP administration of Met-enkephalin and D-Ala^2-Met-enkephalin-NE$_2$ produced faster running and fewer errors in a maze than did the diluent control. Plotnikoff *et al.* [29] administered IP injections of enkephalin to mice and observed increased activity, Tseng *et al.* [30] reported increased analgesia after IV injections of β-endorphin, Olson *et al.* [25] found greater immobilization in goldfish after IP injections of D-Ala^2-β-endorphin but not D-Ala^2-Met-enkaphalin, and Veith *et al.* [31] reported that IP injections of β-endorphin induced grooming in rats. However, even though evidence exists that labeled enkephalin may cross the blood-brain barrier [16], recent findings with a more direct method suggest that the percent may be smaller and more dependent upon concentration than previously realized (Wade, Kastin and Coy, unpublished observations). The evidence for other peptides exerting effects on the brain after peripheral injection has been recently reviewed elsewhere [15].

Accordingly, the purpose of the present study was to compare the effects of intraperitoneal (IP) and intracranial (IC) injections of several neuropeptides on spontaneous activity in goldfish, and to evaluate behaviorally the ability of a substance to permeate the blood-brain barrier, i.e., to enter the brain after being injected peripherally. Goldfish were used because of the ease with which intracranial (IC) and intraperitoneal (IP) injections can be made with the former made into the space over the optic tectum [1,2], because opiate binding sites in the brain have been demonstrated [26], and because the presence of β-lipotropin fragments have been reported in fish [27]. Furthermore, the blood-brain barrier of the goldfish is similar to that found in all higher vertebrates that have been studied, both with regard to site [4] and action [22].

METHOD

Animals

Naive goldfish (*Carassius auratus*) were obtained from Ozard Fisheries, Stoutland, Missouri, and randomly assigned to treatment conditions. The average weight of the 210 fish was 30 g.

Drugs

The peptides were synthesized by solid phase methods [7,8] and dissolved in a vehicle consisting of 0.9% saline

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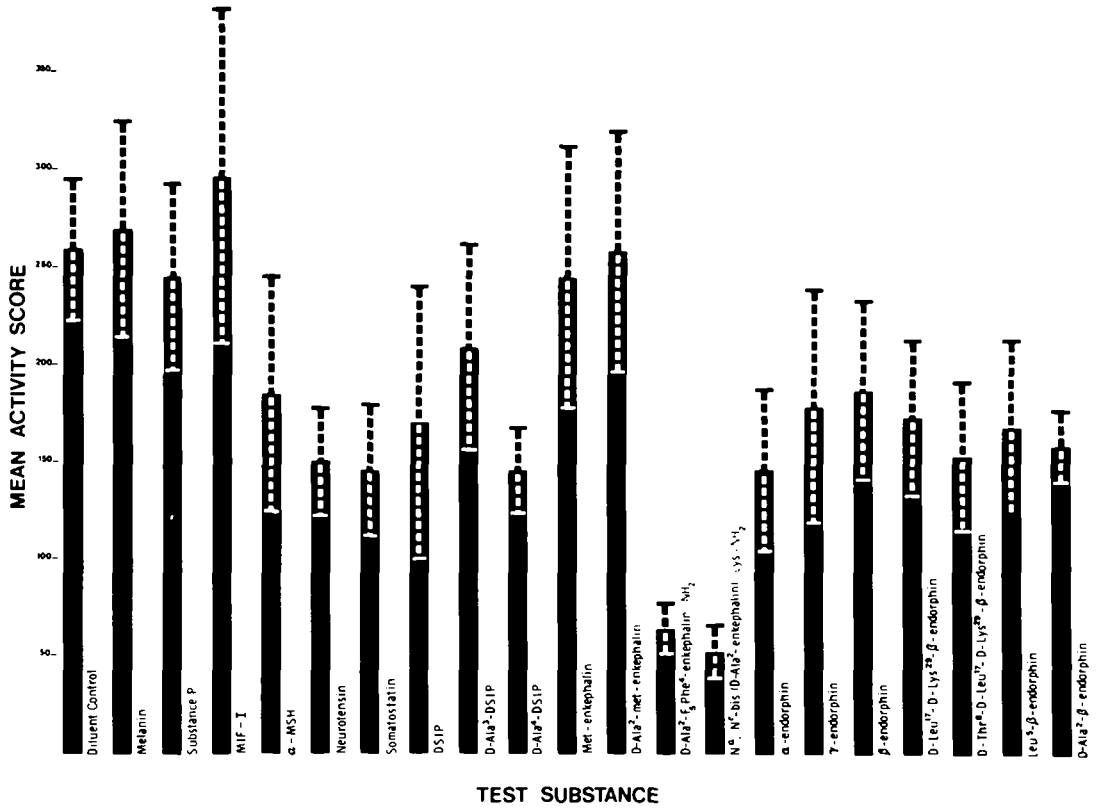

FIG. 1. Mean activity scores for 30 min as a function of test substance.

acidified with acetic acid to 0.01 M, with a pH of 4.1, which also served as the control. A soluble melanin purified from the ink sac of the cuttle-fish (*Sepia officinalis*) was purchased from ICN K and K Rare and Fine Chemical Company, Cleveland, Ohio. Test substances included: diluent, melanin, melanocyte-stimulating hormone (α-MSH), melanocyte-stimulating hormone inhibiting factor (MIF-I), substance P, neurotensin, somatostatin, delta sleep-inducing peptide (DSIP), D-Ala^1-DSIP, D-Ala^5-DSIP, Met-enkephalin, D-Ala^2-Met-enkephalin, D-Ala^2, F$_5$Phe^4-enkephalin-NH$_2$ N$^\alpha$-N$^\epsilon$-bis(D-Ala^2-enkephalin)-Lys-NH$_2$, α-endorphin, γ-endorphin, β-endorphin, D-Leu^{17}, D-Lys^{29}-β-endorphin, D-Thr^6, D-Leu^{17}, D-Lys^{29}-β-endorphin, D-Ala^2-β-endorphin, and Leu^5-β-endorphin.

Apparatus

A standard 10-gal. glass aquarium, containing water 2.5 cm deep, was placed on top of a Stoelting Electronic Activity Monitor (No. 31400). Appropriate programming modules were used to automatically time and record test sessions.

Procedure

Each fish was randomly assigned to one of the 21 treatment groups and received a 5 μl (80 μg/kg) injection of the appropriate substance IP or IC; 5 fish were assigned to each condition. The fish was immediately placed in the apparatus for 30 min with activity scores recorded at the end of each minute. The water was changed after each fish was tested.

RESULTS

The activity scores collected each minute were summed for each fish and the mean activity scores for each of the 21 groups are shown in Fig. 1. A mixed analysis of variance performed on the 21 peptide groups, 2 injection sites, and 30 one-min trials yielded a significant peptide effect, $F(20,168) = 2.046$, $p < 0.01$. Dunnett's test for comparing treatments to a control was used to further analyze the main effect and a significant increase in activity relative to the control was obtained with MIF-I. No reliable differences in activity relative to the control were obtained with substance P, DSIP, melanin, Met-enkephalin, and D-Ala^2-Met-enkephalin. Significant decreases, $p < 0.01$, in activity relative to the control were obtained with the remaining peptides, which are listed by increasing significance: D-Ala^1-DSIP, β-endorphin, D-Leu^{17}, D-Lys^{29}-β-endorphin, γ-endorphin, Leu^5-β-endorphin, D-Ala^2-β-endorphin, D-Thr^6, D-Leu^{17}, D-Lys^{29}-β-endorphin, α-MSH, neurotensin, α-endorphin, somatostatin, D-Ala^1-DSIP, D-Ala^2, F$_5$Phe^4-enkephalin-NH$_2$ and N$^\alpha$N$^\epsilon$-bis(D-Ala^2-enkephalin)-Lys-NH$_2$.

A reliable trials effect, $F(29,4872) = 67.583$, $p < 0.001$, occurred, indicating the decrease in activity over trials. A reliable peptide by trials interaction was also obtained, $F(580,4872) = 1.249$, $p < .0005$, suggesting differential effects of the peptides on activity across trials. A Winer test for simple effects revealed that differential effects for peptides existed only during the initial 6 min of testing.

No other results were significant. That no significant difference existed between injection sites is indicative of the fact that peptides injected at either site had comparable influences on activity.

DISCUSSION

The results of the current study support previous findings showing that peripheral injections of peptides can have significant effect on behavior [14, 15, 17, 18, 22, 25, 29, 30, 31] and further indicate that any differences in the behavioral effects associated with IC and IP injections must be considered a function of the parameters being examined. Although the effects associated with IC injections were more pronounced than the effects after IP injections, the difference was not significant. Thus, the present data differ somewhat from previous studies, including those using goldfish, which demonstrated that IC injections of peptides produce effects reliably greater than peptides injected IP (e.g., [25]), and suggest a greater potential for the clinical use of peptides than previously anticipated. In general, substances administered IC resulted in effects approximately 3 min after injection while substances administered IP required approximately 6 min to produce their effect. This difference in time may well represent the time it takes for a peptide or its metabolite to reach and cross the blood-brain barrier and produce an effect. It also tends to obviate the potential argument that the primary effects of these peptides are exerted in the periphery, for the effects on activity would have been slower rather than faster after IC injections.

A differential effectiveness between test substances was obtained, as expected. However, the differential effects occurred only during the first 6 min of testing, indicating that the action of the peptide was very brief. The effect during this period was highly significant, however, and indicates the potency of the test substances on behavior.

Although only MIF-I produced a reliable increase in activity relative to the control, and this was primarily due to effects associated with IP injections, the finding was consistent with studies done with mice [28], monkeys [9], and humans [10,11]. The effects on activity produced by neurotensin, somatostatin, and α-MSH are compatible with known physiological and behavior results previously reported for these peptides [5, 6, 13, 17]. Although administration of DSIP did not significantly decrease activity, D-Ala^3-DSIP and D-Ala^1-DSIP produced highly significant decreases after both IC and IP injections. The decreased activity as well as the apparent ability of the DSIP analogs to enter the brain is consistent with the recent findings obtained in rabbits by Monnier *et al.* [19, 20, 21].

The greatest decreases in activity were produced by the various forms and analogs of enkephalin and endorphin. The opioid peptides have consistently been reported to decrease and/or immobilize motor activity in a variety of species including fish [25], rats [3,12], and monkeys [24]. Equally consistent has been the finding that β-endorphin or one of its analogs has the most pronounced effects of the various opioid peptides (e.g., [3, 12, 23, 25]). However, data obtained in this study suggest that the flourindated enkephalin analog (D-Ala^2, F$_5$Phe^1-enkephalin-NH$_2$ and the enkephalin dimer (N$^{\alpha}$N$^{\epsilon}$-bis (D-Ala^2-enkephalin)-Lys-NH$_2$) are much more potent in their action on spontaneous activity. The striking effects associated with these two analogs in the current study suggest that they warrant additional evaluation in other paradigms.

Thus, this study provides behavioral confirmation that both IC and IP injections of many peptides may significantly alter behavior with comparable efficacy. Since it has been reported that the blood-brain barrier in goldfish is similar to that found in higher vertebrates with regard to composition and penetration [4,22], it would appear that peripheral injections of some peptides could be effective if a clinical use is established.

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