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Reinforcing Properties of the Neuropeptide Substance P in *Carassius auratus*: Evidence of Dopaminergic System Involvement

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MATTIOLI, R., C. AGUILAR AND L. VASCONCELOS. *Reinforcing properties of the neuropeptide substance P in* Carassius auratus: *Evidence of dopaminergic system involvement.* PHARMACOL BIOCHEM BEHAV **50**(1) 77–81, 1995.—The aim of the present study was to investigate whether neuropeptide substance P (SP) has reinforcing effects in *Carassius auratus* and whether this effect could be related to dopaminergic neurotransmission. For this purpose fishes were put in a three-compartment box in which one compartment gave access to two others that did not directly link. The time spent in each compartment was registered for 10 min to determine a possible preferred compartment. Twenty-four hours later, the fish were given one of the following intraperitoneal treatments: a) Group VEH, injected with the vehicle of substance P; b) Group SP25, injected with SP, 25 μg/kg body wt.; c) Group SP50, injected with SP, 50 μg/kg; or d) Group HALO, injected with haloperidol (2 mg/kg) 30 min before an injection of SP (50 μg/kg). Immediately after the treatment the fish were kept for 30 min in the compartment preferred least the day before. On the next day the fish were retested for 10 min to verify the time spent in each compartment. The results indicate that SP at the dose of 50 μg/kg enhanced the time spent on the paired compartment, and that the pretreatment with haloperidol abolished this enhancement. It is suggested that SP has reinforcing effects in *C. auratus* that may be mediated by the dopaminergic system.

Goldfish Substance P Dopamine Haloperidol Reinforcement Place preference *Carassius auratus*

THE NEUROPEPTIDE substance P (SP) is largely related with learning and memory processes in mammalians. When injected into the lateral hypothalamus and septum, SP facilitated the retention of a previously learned task (39,40); but injected into the substantia nigra or amygdala, it impaired a previously learned task (12,13,39). Peripherally injected SP also improved learning and memory (1,21,22,29,30,43–45). These effects were dose-dependent with an inverted U-shape and occurred only when the injection was made immediately after the training session.

Reinforcing properties of SP have also been postulated. Stäubli and Huston observed a preference in a T-maze for the arm in which the rats previously received SP injection in the lateral hypothalamus and medial septum (41). Using the place-preference test, Huston and Oitzl observed reinforcing properties of SP after intracerebral and intraperitoneal (IP) injections (14). In a further study, using both the traditional place preference and the corral quadrants test, it was demonstrated that SP and its *C*-terminal have reinforcing effects, whereas its *N*-terminus does not (22).

A recent study found that IP injections of SP increase the extracellular concentrations of dopamine (DA) in the neostriatum and nucleus accumbens (3). Moreover, dopaminergic agonists lose their reinforcing effects in rats after the lesion of the nucleus accumbens or DA-antagonists injections into this area (15,34,36). Thus, it is conceivable that the reinforcing effect of SP may be related, at least in part, to the increase of extracellular concentration of dopamine.

As in mammals, there is a wide overlay of dopaminergic cell bodies and SP terminals in the nervous system of *Carassius auratus* (goldfish). This overlay is more evident in the central and medial portions of the telencephalon (11,26,32).

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These telencephalic areas in goldfish are thought to be homologous to the striatum of terrestrian vertebrates (8). Nevertheless, little is known about the functional significance of these areas in teleosts. Lett and Grant were able to show reinforcing effects of amphetamine injections in teleosts from the species *C. auratus* using a place preference test, suggesting that the reinforcing neural system in fishes and mammals have the same evolutionary origin (16).

The place preference paradigm has been widely used to establish reinforcing effects of drugs in many species including the goldfish (16), and was first tested with substances that presented positive reinforcing effects on self-administration studies (5,10,23). In this paradigm the reinforcing effect of a treatment is indicated in two ways: by increasing the amount of time spent in the compartment in which the animal previously was confined after treatment or by a reversal of the place preference shown before the treatment (14).

Considering the overlay between the dopaminergic and SPergic systems in *C. auratus* and the reinforcing effects of dopaminergic agonists in this species, the aims of this work were determine whether SP has reinforcing properties in goldfish, and whether administration of the dopamine antagonist haloperidol (HALO) blocks this effect.

METHOD

Animals

We used 64 experimentally naive *Carassius auratus* (3.5–8.0 g), obtained from a standard source and maintained at 18–22°C in continuously filtered and aerated aquaria of 30 l with 10 fish each. The fish were kept under natural light conditions and fed with basic fish flakes mixed with vegetal fish flakes, 50% each.

Apparatus

A rectangular, three-compartment box was used for the place preference procedure. One white and one black box (22 × 13 cm) were separated by a wall that extended to a gray compartment (8 × 26 cm) that gave access to the other two. The box was filled with 6 cm water at the same temperature as the maintenance aquarium.

Drugs and Treatment

Substance P acetate (Sigma Chemical Co., St Louis, MO) and Haloperidol (Sigma) were dissolved in 6% saline and administered IP in a volume of 0,5 ml/kg body wt. Four treatment groups were formed as follows:

Vehicle (n = 12). Fish were treated with saline just before they were being put into the less-preferred compartment on day 2.

SP 25 (n = 12). Fish were treated with substance P at a dose of 25 μg/kg body wt. on day 2.

SP 50 (n = 18). Fish were treated with substance P at a dose of 50 μg/kg body wt. on day 2 (n = 10), or injected with saline 30 min before the substance P injection (n = 8). The fish pretreated with saline and those treated only with SP were grouped in the same group after we observed that there were no statistical differences between the two groups.

HALO (n = 22). Fish were treated with haloperidol, 2 mg/kg body wt., 30 min before an injection of substance P (50 μg/kg).

Procedure

After an adaptation period to laboratory conditions of 3 weeks, each fish was habituated to the experimental box for 30 min, during two consecutive days. On experimental day 1, each fish was put in the gray compartment and kept in the box for 10 min with free access to all compartments. The time spent in each compartment, as well as the number of entries in each compartment, were recorded. An entry was recorded when the base of the tail crossed the separation line between compartments. Twenty-four hours later (experimental day 2), the fish received the drug treatment, and after 2 min, were put on the less-preferred compartment for 30 min. For this purpose, the compartment was closed with a door that had the same color as the compartment walls. Between injection and placement in the less-preferred compartment, the fish were kept for 2 min in the maintenance aquarium, separated from the others by a Plexiglas wall to reduce stress signs, particularly acceleration of respiratory frequency. This procedure was used to prevent the fish from associating the negative stress state with the compartment. Twenty-four hours later, the fish were tested for place preference. For this purpose, each was put into the gray compartment with free access to the two others and allowed to explore for 10 min; the time spent in each compartment and the number of entries were recorded.

Statistical Analysis

The time spent in each compartment and the number of entries were analyzed using Kruskal-Wallis analysis of variance, with treatment as a factor. Mann-Whitney U test was used for posthoc comparison between treatments. The significance level (5%) was adjusted according to the number of tests used by the "reduced α-method" (17).

RESULTS

The fish showed no initial preference for the black or white compartment before the treatment; 38 preferred the black one, and 25 the white one.

After the treatment and the pairing procedure, the data of the testing day (Fig. 1) indicated that peripheral injection of substance P enhanced the time spent in the previously paired compartment (Kruskal-Wallis, p = 0.0006). The time spent in the paired compartment during the test after the SP injection (50μg/kg) was significantly higher for this group than for the vehicle group (Mann-Whitney U test, p = 0.0072).

The group that was pretreated with haloperidol did not differ from the vehicle group (p = 0.4600) or the group trated with 25 μg/kg SP (p = 0.3122).

The number of entries in each compartment did not differ in any of the groups.

DISCUSSION

The aims of the present study were investigate whether SP had reinforcing properties in goldfish, and whether this effect could be blocked by pretreatment with haloperidol.

The results indicate that SP has reinforcing properties in goldfish at the dose of 50 μg/kg body wt., indicated by a reversal of the initial place preference. Fig. 1 shows an increase in the time spent in the initially less-preferred compartment (paired) with a concomitant decrease in the time spent in the initially preferred compartment (unpaired). Previous studies have indicated that SP at this dose has reinforcing effect in rats, changing the initial preference in a three-compartment-box (14) or increasing the time spent in the drug-paired quadrant in a corral-preference test (9,22). It is difficult to establish homologies between characteristics of two distant

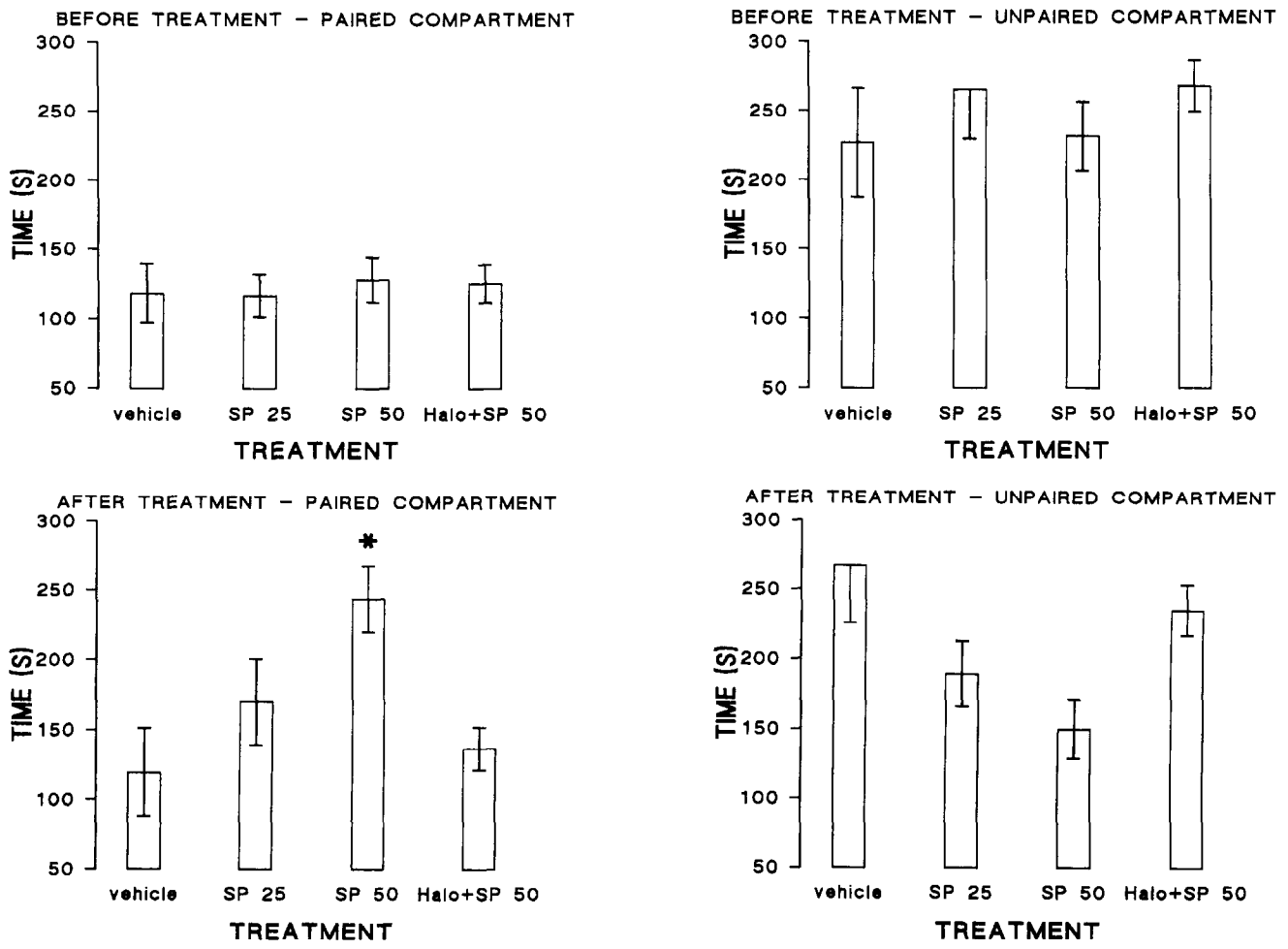

FIG. 1. Mean time (s) spent in each compartment (± SEM). Top left: Paired compartment before treatment. Top right: Unpaired compartment before treatment. Bottom left: Paired compartment after treatment. Bottom right: Unpaired compartment after treatment. *$p < 0.05$, Mann-Whitney U test.

species on the evolutionary scale. Striedter and Northcutt recently proposed that the hypothesis of homology may be argued when there is evidence of similarity in different biologic hierarchic levels, which is strengthened with the addition of new similarities in other hierarchical levels (42). The present results suggest similarities in neural mechanisms underlying learning in fish and rats on two different hierarchic levels, the behavioral and pharmacologic; this supports the hypothesis of homology proposed by Lett and Grant for the central reward system (16). Thus, it is plausible to argue that the basic neural system involved in reward is localized mainly in ancient areas of the central nervous system.

The results of the present study show that pretreatment with haloperidol suppresses the preference for the compartment paired with SP. A role of the mesotelencephalic dopaminergic system in rats has been demonstrated using the place preference paradigm. Various studies indicate that DA-agonists have reinforcing properties (28,33,35), and that the DA-antagonist, haloperidol, widely used for the establishment of DA-mediated behaviors, blocks these effects (6,18,20,27, 31,35,36,37). Regarding haloperidol treatment, it appears that this compound does not disrupt associative processes. Beninger provided considerable evidence that neuroleptics do not block sensory-associative learning (2). In addition, it has been demonstrated that neuroleptics, particularly haloperidol, do not produce conditioned place aversion (5,38). Intraperitioneal injections of SP, as well as its *C*-terminal sequence, have been shown to increase the extracellular concentrations of dopamine (DA) in the neostriatum and nucleus accumbens (3,4), and chronic treatment with substance P has been shown to counteract the effects of lesions to neurons containing dopamine in the substantia nigra of rats (19). Thus, it is possible that the reinforcing property of substance P, blocked by haloperidol, could be mediated at least in part by dopaminergic systems. Because only one dose of substance P was tested after haloperidol treatment, a shift on the dose-response curve cannot be ruled out. Additional studies may be needed to clarify this issue.

Evidence shows that substance P in its original or fragmented form can cross the blood-brain barrier in goldfish; furthermore, the blood-brain barrier of the goldfish is similar to that found in higher vertebrates (24). In rats, two fragments of substance P, the *N*- and *C*-terminals, seem to be involved in different plastic processes. Oitzl, et al. presented evidence

for a positively reinforcing action for substance P and its *C*-terminal sequence when administered systemically, but not for its *N*-terminus (22). Otherwise, it seems that the effects of SP on mnemonic processing are mediated by its *N*-terminal part (25). Although no data are available on the stability of substance P on fish, it was found that in rat blood plasma, 50% of substance P is inactivated in 12 min (7). Therefore, it is possible that the reinforcing effect of SP in fish observed in this study mainly results from the effects of its *C*-terminal. Further research is needed to isolate the relative contribution of SP fragments to the reinforcing mechanisms in goldfish. In addition, selective lesions of brain areas of goldfish containing SP terminals and DA cell bodies could indicate phylogenic ancient circuits of reinforcement, learning, and memory.

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REFERENCES

1. Aguiar, M. S.; Tomaz, C. Enhanced habituation produced by posttrial peripheral injection of substance P. Bull. Psychonomic Soc. 28:204–106; 1990.
2. Benninger, R. J. The role of dopamine in locomotor activity and learning. Brain Res. Rev. 7:173–195; 1983.
3. Boix, F.; Mattioli, R.; Adams, F.; Huston, J. P.; Schwarting, R. K. W. Effects of substance P on extracellular dopamine in neostriatum and nucleus accumbens. Eur. J. Pharmacol. 216: 103–107; 1992.
4. Boix, F.; Huston, J. P.; Schwarting, R. K. W. The *C*-terminal fragment of substance P enhances dopamine release in nucleus accumbens but not in neostriatum in freely moving rats. Brain Res. 592:181–186; 1992.
5. Bozarth, M. A.; Wise, R. A. Intracranial self-administration of morphine into the ventral tegmental area in rats. Life Sci. 28:551–555; 1981.
6. Bozarth, M. A.; Wise, R. A. Heroin reward is dependent on dopaminergic substrate. Life Sci. 29:1881–1886; 1981.
7. Couture, R.; Regoli, D. Inactivation of substance P and its C-fragments in rat plasma and its inhibition by Captopril. Can. J. Physiol. Pharmacol. 59:621–625; 1981.
8. Echteler, S. M.; Saidel, W. M. Forebrain connections in the goldfish support homologies with land vertebrates. Science 212:683–685; 1981.
9. Hasenöhrl, R. V; Gerhhardt, P.; Huston, J. P. Evidence for dose-dependent positively and negatively reinforcing effects of the substance P *C*-terminal analog DIME-C7. Neuropeptides 17: 205–211; 1990.
10. Hoebel, B. G.; Monaco, A. B.; Hernandez, L.; Aulisi, E. F.; Istanley, B. G.; Lenard, L. Self-injection of amphetamine directly into the brain. Psychopharmacology (Berlin) 81:158–163; 1983.
11. Hornby, P. J.; Piekut, D. T.; Demsky, L. S. Localization of immunoreactive tyrosine hydroxylase in the goldfish brain. J. Comp. Neurol. 261:1–14; 1987.
12. Huston, J. P.; Stäubli, U. Retrograde amnesia produced by posttrial injection of substance P into substantia nigra. Brain Res. 159:468–472; 1978.
13. Huston, J. P.; Stäubli, U. Posttrial injection of substance P into lateral hypothalamus and amygdala, respectively, facilitates and impairs learning. Behav. Neural Biol. 27:244–248; 1979.
14. Huston, J. P.; Oitzl, M.-S. The relationship between reinforcement and memory: Parallels in the rewarding and mnemonic effects of the neuropeptide Substance P. Neurosci. Biobehav. Rev. 13:171–180; 1989.
15. Kurumiya, S.; Nakajima, S. Dopamine D_1 receptors in the nucleus accumbens: Involvement in the reinforcing effect of tegmental stimulation. Brain Res. 448:1–6; 1988.
16. Lett, B. T.; Grant, V. L. The hedonic effects of amphetamine and pentobarbital in goldfish. Pharmacol. Biochem. Behav. 32: 355–356; 1989.
17. Lunney, G. H. Individual size for multiple T-tests. Am. Ed. Res. J. 6:701–703; 1969.
18. Martin-Iverson, M. T.; Ortman, R.; Fibinger, H. C. Place preference conditioning with methylphenidate and nomifensine. Brain Res. 332:59–67; 1985.
19. Mattioli, R.; Schwarting, R. K. W.; Huston, J. P. Recovery from unilateral 6-hydroxidopamine lesion of substantia nigra promoted by the neurotachykinin substance P 1–11. Neuroscience 48:595–605; 1992.
20. Mithani, S.; Martin-Iverson, M. T.; Phillips, A. G. The effects of haloperidol on amphetamine- and methylphenidate-induced conditioned place preferences and locomotor activity. Psychopharmacology 90:247–252; 1986.
21. Nogueira, P. J. C.; Tomaz, C. Substance P and naloxone enhancement of avoidance conditioning in rats. Brazil. J. Med. Biol. Res. 23:163–167; 1990.
22. Oitzl, M.-S.; Hasenöhrl, R. U.; Huston, J. P. Reinforcing effects of peripherally administered substance P and its *C*-terminal pGlu6-SP 6–11 in the rat. Psychopharmacology 100:308–315; 1990.
23. Olds, M. E.; Williams, N. K. Self-administration of D-ala2-metenkephalinamide at hypothalamic self-stimulation sites. Brain Res. 194:155–170; 1980.
24. Olson, R. D.; Kastin, A. J.; Montalbano-Smith, D.; Olson, G. A.; Coy, D. H.; Michell, G. F. Neuropeptides and the blood-brain barrier in goldfish. Pharmac. Biochem. Behav. 9:521–524; 1978.
25. Pelleymounter, M. A.; Fisher, Q.; Schlesinger, K.; Hall, M.; Dearmey, P.; Stewart, J. The effect of substance P and its fragments on passive avoidance retention and brain monoamine activity. Behav. Brain Res. 21:119–127; 1986.
26. Peter, R. E.; Gill, V. E. A stereotaxic atlas and technique for forebrain nuclei of the goldfish Carassius auratus. J. Comp. Neurol. 159:69–102; 1975.
27. Phillips, A. G.; LePiane, F. G.; Fibinger, H. C. Dopaminergic mediation of reward produced by direct injection of enkephalin into the ventral tegmental area of the rat. Life Sci. 33:2505–2511; 1983.
28. Reicher, M. A.; Holman, E. W. Location preference and flavor aversion reinforced by amphetamine in rats. Anim. Learn. Behav. 5:343–346; 1977.
29. Schlesinger, K.; Lipsitz, D. U.; Peck, P. L.; Pelleymounter, M. A. Substance P reversal of electroconvulsive shock and cycloheximide-induced retrograde amnesia. Behav. Neural Biol. 39:30–39; 1983.
30. Schlesinger, K.; Pelleymounter, M. A.; Van de Kamp, J.; Bader, D. L.; Stewart, J. M.; Chase, T. N. Substance P facilitation of memory: Effects in an appetitively motivated learning task. Behav. Neural Biol. 45:230–239; 1986.
31. Schwartz, A. S.; Marchok, P. L. Depression of morphine-seeking behaviour by dopamine inhibition. Nature 248:257–258; 1974.
32. Sharma, S. C.; Berthoud, V. M.; Breckwoldt, R. Distribution of substance P-like immunoreactivity in the goldfish brain. J. Comp. Neurol. 279:104–116; 1989.
33. Sherman, J. E.; Roberts, T.; Roskam, S. E.; Holman, E. W. Temporal properties of rewarding and aversive effects of amphetamine in rats. Pharmacol. Biochem. Behav. 13:597–599; 1980.
34. Shippenberg, T. S.; Bals-Kubic, R.; Huber, A.; Herz, A. Neuroanatomical substrates mediating the aversive effects of D_1 dopamine receptor antagonists. Psychopharmacology 103:209–214; 1991.
35. Spyraki, C.; Fibinger, H. C.; Phillips, A. G. Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Res. 253:185–193; 1982.
36. Spyraki, C.; Fibinger, H. C.; Phillips, A. G. Cocaine-induced

place preference conditioning: Lack of effects of neuroleptics and 6-hydroxidopamine lesions. Brain. Res. 253:195-203; 1982.
37. Spyraki, C.; Fibinger, H. C.; Phillips, A. G. Place preference conditioning using food reinforcement: Attenuation by haloperidol. Psychopharmacology 77:379-382; 1982.
38. Spyraki, C.; Fibinger, H. C.; Phillips, A. G. Attenuation of heroin reward in rats by disruption of the mesolimbic dopamine system. Psychopharmacology 79:278-283; 1983.
39. Stäubli, U.; Huston, J. P. Differential effects on learning by ventromedial vs. lateral hypothalamic posttrial injection of substance P. Pharmacol. Biochem. Behav. 10:783-786; 1979.
40. Stäubli, U.; Huston, J. P. Facilitation of learning by posttrial injection of substance P into the medial septal nucleus. Behav. Brain Res. 1:245-255; 1980.
41. Stäubli, U.; Huston, J. P. Central action of substance P: possible role in reward. Behav. Neural. Biol. 43:100-108; 1985.
42. Striedter, G. F.; Northcutt, R. G. Biological hierarchies and the concept of homology. Brain Behav. Evol. 38:177-189; 1991.
43. Tomaz, C.; Huston, J. P. Facilitation of conditioned inhibitory avoidance by posttrial peripheral injection of substance P. Pharmacol. Biochem. Behav. 25:469-472; 1986.
44. Tomaz, C.; Aguiar, M. S.; Nogueira, P. J. C. Facilitation of memory by peripheral administration of substance P and naloxone using avoidance and habituation learning tasks. Neurosci. Biobehav. Rev. 14:447-453; 1990.
45. Wetzel, W.; Matthies, H. Effect of substance P on the retention of a brightness discrimination task in rats. Acta Biol. Med. Germ. 41:647-652; 1982.