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Effect of Brain Peptides on Hypokinesia Produced by Anterolateral Hypothalamic 6-OHDA Lesions in Rats

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RONDEAU, D. B., F. B. JOLICOEUR, F. BELANGER AND A. BARBEAU. *Effect of brain peptides on hypokinesia produced by anterolateral hypothalamic 6-OHDA lesions in rats.* PHARMAC. BIOCHEM. BEHAV **10**(6) 943–946, 1979.—Intraventricular injections of substance P, TRH and somatostatin were administered to rats rendered hypokinetic by bilateral microinjections of 6-hydroxydopamine into the anterolateral hypothalamus. Only substance P in a dose of 0.30 μg/rat significantly increased motor activity as determined by photocell counts in a 5 min test session immediately after administration of the peptide. Behavioral observations indicated that grooming and not locomotion was mainly responsible for the greater activity scores. None of the three peptides at the doses examined potentiated or reduced the increased activity induced by 1 mg/kg apomorphine. Stereotyped behavior was also not affected by previous injections of substance P and somatostatin but was enhanced in animals which had received 5 μg/rat TRH 30 min prior to apomorphine.

Substance P TRH Somatostatin 6-Hydroxydopamine Motor activity Hypokinesia Apomorphine

BILATERAL microinjections of 6-hydroxydopamine (6-OHDA) in the anterolateral hypothalamus has been proposed as an experimental model of the hypokinesia of Parkinson's disease [22]. It has been demonstrated that the hypokinesia, which is accompanied by a generalized reduction in brain noradrenaline levels and a reduction of dopamine in the striatum and cerebral cortex [3], is temporarily reversed following the administration of several drugs used in the treatment of parkinsonism, such as Piribedil, Bromocriptine and L-Dopa, alone [9] or in combination with peripheral decarboxylase inhibitor, Ro4-4602 [3]. The putative dopamine receptor agonist apomorphine is also effective in reversing the hypokinesia produced by the bilateral 6-OHDA lesions [3,18]. In agreement with other animal studies which had shown that the tripeptide L-prolyl-L-leucyl-glycine-amide (PLG or MIF) potentiates some behavioral effects of L-Dopa [11,16], Barbeau and Kastin [2] found that the effect of apomorphine upon motility in the hypokinetic 6-OHDA lesioned rats was potentiated by the peripheral administration of PLG. Although PLG alone did not reverse the hypokinesia, its combination with apomorphine resulted in motility scores significantly greater than those obtained following apomorphine treatment alone. Recently, a similar finding on rotational behavior in rats with striatal 6-OHDA lesions confirmed the potentiation of apomorphine action by PLG [14]. The purpose of the present experiment was to determine the effects of intraventricular administration of three other peptides, substance P (SP), Thyrotropin-releasing hormone (TRH) and somatostatin alone and in combination with apomorphine in rats rendered hypokinetic by bilateral 6-OHDA hypothalamic lesions. Results demonstrate that only SP at a dose of 0.30 μg/rat increased motor activity and that only TRH at a dose of 5.0 μg/rat potentiated the stereotypy produced by apomorphine in these rats.

METHOD

Animals

Male Sprague-Dawley rats obtained from Canadian Breeding Farm (St-Constant, P. Québec) were used. They were housed in a temperature and humidity controlled room having a 12 hr light-dark cycle. Food (Purina Rat Chow) and water were available ad lib. Rats weighed 275–325 g at surgery.

Surgery

Surgical procedures were carried out in rats anesthetized with sodium pentobarbital (60 mg/kg) using a David Kopf stereotaxic instrument. Each microinjection consisted of 4 μl of distilled water containing 6-hydroxydopamine (Sigma Chemicals), 6.5 μg/μl and ascorbic acid, 0.4 μg/μl. Injection solutions were mixed just prior to operation and chilled until loaded into a 10 μl Hamilton syringe (No. 701) fitted with a shortened (3 cm) needle; rate of injections was 1 μl/min. Rats were injected bilaterally in the anterolateral region of the hypothalamus, 7.0 mm anterior to the interaural line, 2.0 mm lateral to the sagittal sinus and 8.0 mm ventral to the dural surface; stereotaxic coordinates of the microinjections of

6-OHDA were based on the atlas by De Groot [8] Animals were chronically implanted with a cannula into the left ventricle immediately after 6-OHDA administration and placed in individual cages. Behavioral measures were taken 48 hours after surgery.

Procedure

Motor activity was measured using a circular (70 cm) activity meter (Lehigh Valley Electronics) comprising 6 photoelectric cells. Activity scores were recorded for 5 min at 10 min intervals. There were 10, 5 min test sessions in the activity meter. Behavioral observations were made during the sessions and the incidence of grooming, hypersalivation, forward locomotion and motor disturbances was noted. No treatment was administered before the first test session which constituted an adaptation period to the activity meter. Rats received intraventricular (IVT) injections of various doses of substance P (SP), somatostatin, thyrotropin releasing hormone (TRH) or their respective vehicle immediately prior to the second test session. Subcutaneous (SC) injections of 1 mg/kg apomorphine (Apomorphine HCl, MacFarlan-Smith) were given immediately prior to the fourth test session. Thus, in all cases, IVT injections of the peptides or their vehicle preceded by 30 min apomorphine administration. Upon removal from the apparatus after completion of a test session rats were placed in wire-mesh cages for the next 10 min and observed for stereotypy. Stereotyped behavior was rated according to the scale used by Costall *et al.* [7]: 0 - no stereotyped behavior; 1 - periodic sniffing and/or repetitive head and limb movements; 2 - continuous sniffing and/or repetitive head and limb movements: 3 - periodic gnawing, biting or licking: 4 - continuous gnawing, biting or licking.

Synthetic SP (Peninsula Laboratories, San Carlos, California) was dissolved in a 0.01 N acetic acid solution previously buffered with NaOH to pH =6.2. Six groups of 8 rats were used. One group of animals was injected with the vehicle only and served as a control group. The other groups corresponded to the following doses of SP: 0.07, 0.30, 1.25, 5.00 and 20.00 μg. The injection volume was 20 μl: it was administered over a 2 min period via a 50 μl Hamilton syringe. Only 6 animals of each group were tested following 1 mg/kg apomorphine SC.

TRH and somatostatin (Peninsula Laboratories, San Carlos, California) were dissolved in 0.9% NaCl. Eight groups of 6 rats were used for each peptide. The following doses were administered in a volume of 20 μl: 0.00, 1.25, 2.50 and 5.00 μg. Animals were then tested following 1 mg/kg apomorphine SC

Within 48 hours after the behavioral testing was completed, animals were killed by cervical dislocation Brains were rapidly removed, placed in Formalin (10%) for at least 48 hours and then sectioned (40 μ) on a freezing microtome. Needle tracks were clearly visible indicating the site of 6-OHDA injection. Data collected on animals for which the site of 6-OHDA injection did not match the stereotaxic coordinates mentioned above were discarded.

RESULTS

Microinjections of 6-OHDA produced aphagia and adipsia In each group, rats suffered body weight losses ranging from 25 to 45 g during the 48 hr period following the bilateral anterolateral hypothalamic injections. All animals displayed

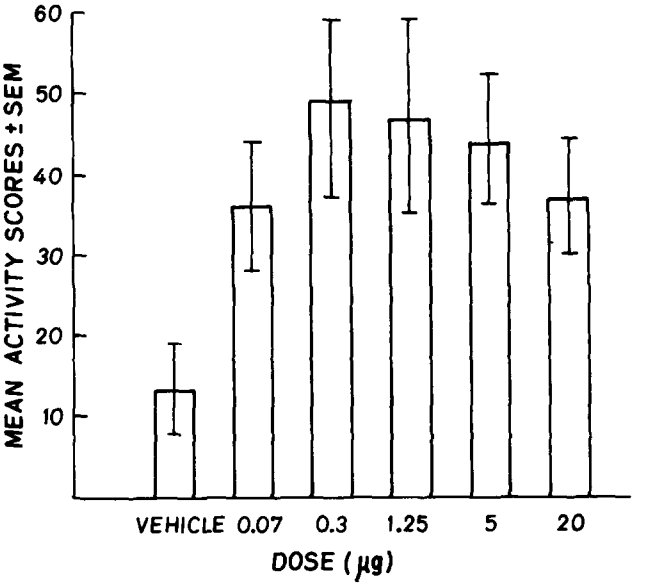

FIG. 1 Mean activity scores during the 5 min test session following intraventricular administration of substance P in 6-OHDA treated rats Eight rats per group.

hypokinesia. Mean activity scores for all groups treated with 6-OHDA during the first 5 min test session ranged from 10.8 to 29.6 counts while sham-lesioned or naive rats steadily presented scores of over 100 counts. Activity scores of the various groups which received IVT injections of SP prior to the second test session were analysed by means of a one-way ANOVA [25]. A post hoc Dunnett test indicated that the activity score for the group administered the 0.30 μg dose of SP was significantly greater than that of the control group ($p<0.01$). This is illustrated in Fig. 1 in which means activity scores during the 5 min test session are plotted for each dose of SP. Behavioral observations revealed that the significantly greater activity in the 0.30 μg SP group as well as the higher mean activity counts in other groups which received SP were not necessarily related to the induction of locomotor activity in the hypokinetic 6-OHDA treated rats. Grooming was observed in all animals injected with 0.30 μg SP while only 3 out of 8 rats showed locomotor activity. Whenever observed after IVT administration of SP, locomotion was slow and consisted of extremely short steps. All rats were hypokinetic during the third test session which took place 15 min after injection of SP; activity scores were similar to those recorded during the first test session, indicating that the various effects of SP, including the increased activity produced by the 0.30 μg dose, were of short duration.

Separate one way ANOVAs and the appropriate post hoc tests revealed that IVT injections of TRH and somatostatin, in doses of 1 25, 2.50 and 5.00 μg did not produce an increase in motor activity during the second test session No significant differences were found between activity scores during the third test session in the TRH and somatostatin groups respectively.

Three separate one way analyses of variance were carried out on the total activity scores of the 7 test sessions which followed apomorphine administration, sessions 4 to 10. SP, TRH and somatostatin at the doses examined did not potentiate or reduce the effects of 1 mg/kg apomorphine SC on

motor activity in 6-OHDA treated rats. The latency, intensity and duration of the apomorphine induced stereotyped behavior was not significantly affected in the animals previously injected with SP and somatostatin. However, Fisher exact probability tests [21] determined that the 5.00 μg TRH group differed significantly from the control group in the proportion of animals which displayed the low (scores of 1 or 2) and the high (scores of 3 and 4) components of stereotyped behavior ($p<0.01$). This significant difference was obtained only for a period extending from 30 to 45 min after apomorphine administration. Although intensity of stereotyped behavior was greater in the animals which received 5.00 μg, TRH injections latency and duration of the stereotypy remained unaffected. The two other doses of TRH did not modify significantly stereotyped behavior.

DISCUSSION

Two days following bilateral 6-OHDA lesions at the level of the anterolateral hypothalamus, rats show a marked decrease in spontaneous motor activity usually referred to as hypokinesia [3,22]. Results of the present experiment indicate that IVT injections of 0.30 μg/rat SP reverse temporarily this hypokinesia during a 5 min test session in an activity meter. However, the reversal of the hypokinesia by 0.30 μg/rat SP was of much less magnitude and duration than the one produced by the administration of apomorphine, L-Dopa or other dopaminergic agonists [3]. SP induced very little locomotor activity in the hypokinetic animals and grooming movements appeared to be mainly responsible for the increased activity scores. When locomotion was observed, gait of rats consisted of extremely short steps, executed very slowly. Such abnormal walking has been reported to occur following injections of anticholinergics in akinetic 6-OHDA treated rats [20]. Data obtained from this laboratory have shown that the 0.30 μg/rat dose of SP also significantly increased motor activity in naive animals previously adapted to an identical test environment; grooming was observed, but animals did not significantly spend more time than control rats in such activity [19]. Behavioral excitation characterized by sniffing and hypermotility as well as increased motor activity and grooming have been reported following bilateral injections of SP into the ventral tegmental area [23] and into the zona reticulata of the substantia nigra [23] respectively. The first indirect evidence that SP may exert an excitatory action on dopaminergic neurons had been provided by the observation that unilateral intranigral application of SP caused contralateral rotations in rats [12,15]. In the present experiment SP was administered into the ventricules and it cannot be established precisely upon which brain structures the peptide might have exerted its action. Intraventricular injections of SP have been reported to stimulate the formation of dopa in various brain regions, including the striatum and the limbic system [4].

Lower and higher doses of the peptide did not significantly change motor activity during that period. Hypersalivation for which a wet fur following an episode of grooming was used as a crude index, was seen in a majority of animals in the 0.30, 1.25 and 5.0 μg SP groups. The highest dose of SP examined, 20 μg/rat, produced motor disturbances characterized by abnormal postures, head tremors and rigidity in 5 out of 8 rats; barrel rolling rotations were seen in two of these animals. Rotational movements after IVT administration of SP in doses ranging from 20–100 μg/rat have been described previously [4,19].

TRH and somatostatin did not reverse the hypokinesia of the 6-OHDA lesioned animals. Behavioral observations indicated that grooming, hypersalivation, locomotion and motor disturbances did not occur in the rats injected with TRH. However repetitive shaking of the body, that is the so-called "wet dog shake behavior" was noted in 4 out of 6 rats in the 2.50 and 5.00 μg TRH groups. Less than 10 wet dog shakes were recorded during the test session. Abnormal postures and gross motor disturbances were frequently observed following IVT injections of somatostatin. The severity of these disturbances appeared to be dose related. The response to the administration of the 1.25 and 2.50 μg doses of somatostatin was characterized by a flattened rigidity with extension of the limbs interrupted by jerks, dystonic contractions and rolling movements from side to side; at the higher dose, the latter movements culminated into several barrel rolling rotations in 4 out of 6 animals. The motor disturbances occurred within few seconds after completion of the injections of somatostatin; animals were completely immobile during the last minute of the session in the activity meter. The wet dog shaking behavior seen after TRH injections and the abnormal postures, rigidity, dystonic movements and, at higher doses, the accompanying barrel rolling rotations which were observed immediately after IVT administration of somatostatin have been shown to occur in naive rats [5, 10, 24].

Administration of apomorphine, 1 mg/kg SC, clearly reversed for a period of approximately 1 hr the hypokinesia resulting from the 6-OHDA hypothalamic lesions. Unlike what has been found for PLG [2,14], the increased activity produced by apomorphine was not potentiated by SP, TRH and somatostatin. Moreover, SP and somatostatin did not affect apomorphine induced stereotyped behavior. It was accentuated only in animals previously injected with 5.00 μg/rat TRH; all rats in this group displayed the higher components of stereotypy, biting or licking, 30 min after apomorphine injection. Previous reports have indicated that IVT injection of SP did not modify motor activity and stereotyped behavior in non lesioned animals pretreated with apomorphine (1 mg/kg SC) [19], while central administration of high doses of TRH and somatostatin produced respectively tight head to tail rotations and barrel rolling rotations in rats pretreated with apomorphine [5,6].

Biochemical, morphological and histochemical studies have led to the recognition of the existence of peptidergic neurons and pathways. There is rapidly increasing evidence that brain peptides, including SP, TRH and somatostatin, play physiological roles in neuronal and behavioral functions of the CNS (for review, [17]) possibly by modulating directly or indirectly neurotransmission. It has been hypothesized that an important role of the peptidergic pathways is to exert a trophic modulation on aminergic functions and that a decrease in such trophic action of peptides could result in damages to neurons containing the putative neurotransmitters, like those associated with extrapyramidal disorders [1]. As evidenced by the clinical utilization of PLG [1,2] and TRH [17], valuable information concerning the pathophysiology and/or therapy of neurological disorders can be obtained from the study of the relationship between brain peptides and biogenic amines.

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