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Research report

The effect of thrombin on a 6-hydroxydopamine model of Parkinson's disease depends on timing

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Abstract

Recent results in animal models suggest that thrombin may modulate brain injury in Parkinson's disease (PD). High doses of thrombin (~20 U) can damage dopaminergic neurons, while we have found that low dose thrombin (1 U), given several days before a brain insult (thrombin preconditioning), is protective in models of PD and stroke. However, the effects of such low levels of thrombin at the time of, or after, exposure to the dopamine neurotoxin 6-hydroxydopamine (6-OHDA) have not been examined and are the focus of this study. In the first set of experiments, rats received co-administration of thrombin (1 U) or saline and 6-OHDA (5 μg) into the medial forebrain bundle. 6-OHDA + thrombin resulted in striking increases in behavioral deficits, compared to 6-OHDA + saline. Similarly, co-administration of an agonist to protease-activated receptor (PAR)-1, a thrombin receptor, also resulted in significantly greater behavioral deficits. In a second set of experiments, thrombin (1 U) or saline was administered 1 or 7 days after 6-OHDA to determine the effects of thrombin after 6-OHDA. Surprisingly, the rats that received saline had strikingly increased behavioral and neurochemical deficits resulting from the 6-OHDA lesion, while delayed thrombin administration prevented this effect. The results indicate that thrombin has differential effects in the 6-OHDA model, dependent on the time of administration. The ability of a second cannula insertion with saline infusion to increase dramatically deficits raises questions as to what role physical injury to already susceptible cells might play in the pathogenesis of some cases of PD.

Keywords: Parkinson's disease; Brain injury; Sensorimotor; Protease-activated receptor-1; PAR-1

1. Introduction

Thrombin derived from blood or brain prothrombin has been implicated in modulating brain injury in hemorrhagic and ischemic stroke [46]. Thrombin is a key mediator of edema formation and cell death in experimental stroke [26,37,41]. Recent evidence also suggests that thrombin may modulate brain injury in Parkinson's disease (PD). High doses of thrombin (~20 U) can lead to degeneration of dopaminergic neurons in the substantia nigra, although cell-type specificity is still unclear [5,9]. This effect is likely mediated by cell death pathways involving microglial activation [5,8,10,25]. It is also interesting that intracerebral hemorrhage (with resultant thrombin production) in or near the nigrostriatal tract, has

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been found to produce L-DOPA responsive behavioral deficits similar to those observed in PD [19,30]. Many thrombin effects are receptor mediated, involving protease-activated receptor (PAR) activation [21]. Mice deficient in PAR-1 are protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced neurotoxicity [16], which causes PD-like effects in humans and animals [23,24].

In contrast to the detrimental effects of high doses of thrombin, administration of a low dose thrombin several days prior to experimental stroke is highly protective, an effect termed thrombin preconditioning [29,45]. It is well established that a minor insult to the brain can activate cell survival mechanisms that can reduce or prevent tissue destruction normally expected from a severe insult several days later [15]. In ischemia models, resistance to an otherwise major cereberovascular challenge has been called "ischemic tolerance". Ischemic or thrombin preconditioning does not produce detectable tissue loss, although sensitive behavioral assessment suggests that they may cause transient functional deficits reflecting sub-fatal trauma, which may be sufficient to prepare the brain for more severe insults [3,18].

Recently, a low dose of thrombin (1 U) was also found to be protective in a 6-hydroxydopamine (6-OHDA) model of experimental PD [3]. Such cross-tolerance among other injury models is known to occur [15]. Although the mechanisms of protection remain to be determined, the preconditioning effects of thrombin depend on activation of the protease-activated receptor-1 (PAR-1) [4], a thrombin receptor linked to injury tolerance [21].

These findings suggest a dose and a temporal relationship in the effects of thrombin in neurological disease models. In considering the potential of thrombin to contribute to the pathogenesis of PD it is, therefore, important to determine the effects of both co- and post-administration of thrombin in experimental PD models. The current study, therefore, examined the effects of co-administering a low dose of thrombin (1 U) or a PAR-1 agonist with the neurotoxin 6-OHDA. 6-OHDA-induced neurotoxicity in the rat is a commonly used experimental model of PD. The study also examined the effects of thrombin administration at different time points after 6-OHDA. There were differential effects on behavioral and neurochemical endpoints based on administration time. Co-administration of either thrombin or a PAR-1 agonist at the time of the neurotoxin delivery increased behavioral and neurochemical deficits. Interestingly, a second cannula insertion and infusion of saline after 6-OHDA potentiated injury, while thrombin prevented that effect.

2. Materials and methods

2.1. Materials

Unless otherwise noted, all chemicals were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA).

2.2. Animals

All animal protocols were approved by the University of Michigan Committee on the Use and Care of Animals. For all experiments, adult male Sprague–Dawley rats (250–325 g; Charles River Laboratories, Portage, MI, USA) were used. Rats were allowed free access to food and water. All animals were housed in a temperature controlled room (25 °C) with a 12:12 h light:dark cycle. Behavioral tests were typically conducted in the morning near the beginning of the light cycle.

2.3. Surgery

Prior to surgery, animals were fully anesthetized with pentobarbital (50 mg/kg, Abbott Laboratories, North Chicago, IL, USA). Rats were then placed in a stereotaxic frame (Model 5000, Kopf Instruments, Tujunga, CA, USA). A longitudinal incision was made in the scalp, the skull was exposed, and then a cranial burr hole (1 mm) was drilled into the skull at the infusion site. A 26-gauge Hamilton syringe needle (Hamilton Company, Reno, NV, USA) was then lowered into the right medial forebrain bundle (anterior/posterior: −2.2 mm, right/left: 1.9 mm, ventral: 8.0 mm to bregma) and the animals were administered the drug treatments. For those animals that received a second surgery, the procedure was conducted as described above, except a 30-gauge syringe needle was used and the infusion occurred 1 mm dorsal to the site of the first infusion, in order to account for the larger volume (50 μL). Bone wax was used to fill the burr hole and the surgery site was stitched after the infusion. After completion of each infusion, the cannula was allowed to remain in place for 5 min to allow for proper absorption and avoid cortical exposure during cannula withdrawal. Core body temperature was maintained at 37 °C during the surgery and until the animals were ambulatory, using a rectal probe feedback-controlled heating pad.

For 6-OHDA infusions, 30 min prior to lesioning animals received the noradrenergic uptake blocker, desipramine (15 mg/kg, i.p.) and the monoamine oxidase inhibitor pargyline (25 mg/kg, i.p.) to enhance dopaminergic neurotoxicity, and limit the loss of noradrenergic neurons [36].

2.4. Experimental design

This study was divided into three parts. In part I, the effects of different doses of 6-OHDA on behavioral deficits were examined. We have previously used a dose of 10 μg to examine the protective effects of thrombin preconditioning [3]. However, that 6-OHDA dose produces near maximal deficits in the behavioral tests used. Since we expected that thrombin might enhance deficits in some experiments, we examined the behavioral deficits with a lower dose (5 μg; $n = 5$) in comparison to that found with 10 μg ($n = 9$), to ensure that the lower dose would lead to detectable, but not maximal, deficits. The total infusion volume was 4 μL and occurred over 8 min.

In part II, the effects of thrombin or a PAR-agonist, when co-administered with 6-OHDA were tested. 6-OHDA (5 μg) and either saline ($n = 11$), thrombin (1 U; $n = 5$), or a PAR-1 agonist [$n = 6$; 5 nmol; Ala-para-fluorPhe-Arg-Cha-homoArg-Tyr-NH_2; NeoMPS, Strasbourg, France; [43]] were infused into the right medial forebrain bundle. The total infusion volume for these studies was 6 μL and occurred over 12 min.

In part III, the effects of thrombin when administered after 6-OHDA were examined. 6-OHDA (5 μg) was administered as described above. The total infusion volume was 4 μL and occurred over 8 min. A second infusion of either saline (50 μL; $n = 5$) or thrombin (1 U in 50 μL saline; $n = 6$) was administered 7 days after 6-OHDA. This concentration of thrombin was chosen because it has previously been found to be protective when administered prior to 6-OHDA [3]; it is, therefore, ideal to further elucidate the temporal effects of thrombin in the 6-OHDA model. Additionally, this dosage of thrombin (1 U/50 μL saline) does not produce brain edema [45]. To evaluate the effects of thrombin at an earlier time-point after 6-OHDA, additional animals were administered either saline ($n = 4$) or thrombin ($n = 5$) 1 day after 6-OHDA.

2.5. Neurobehavioral assessment

All animals in these studies underwent behavioral analysis prior to and through 14–21 days after 6-OHDA administration. The observer was blinded to the treatment group until completion of all behavioral studies.

2.5.1. Vibrissae-elicited forelimb placing (placing test)

This test has been well described and utilized to measure 6-OHDA-elicited deficits [35,39,44]. Additionally, thrombin preconditioning has been found to provide protection from 6-OHDA-elicited deficits in this test [3].