A Substance P Antagonist, [D-Pro^2, D-Trp^{7,9}]SP, Inhibits Inflammatory Responses in the Rabbit Eye

Abstract. *Neurogenic factors released by antidromic nerve stimulation are thought to be in part responsible for the vasodilation and breakdown of the blood-aqueous barrier that follows trauma to the eye. Substance P is one candidate for the mediation of the inflammatory response since it is thought to be a neurotransmitter in sensory afferents and since exogenous substance P is capable of eliciting a response characteristic of inflammation. In rabbits, intravitreal or topical application onto the eye of a specific substance P antagonist, [D-Pro^2, D-Trp^{7,9}]SP, inhibited not only the irritant effects of exogenous substance P but also the inflammatory response to a standardized trauma (infrared irradiation of the iris). These observations suggest that substance P, or a related peptide, is a neurogenic mediator of the inflammatory response in the eye.*

The inflammatory response to trauma in the eye, characterized by constriction of the pupil (miosis), hyperemia, and breakdown of the blood-aqueous barrier, is thought to involve antidromic stimulation of sensory nerve fibers since treatment with the neuronal blocking agent tetrodotoxin or trigeminal denervation prevents or greatly reduces the response (*1, 2*). Studies on skin suggest that both vasodilation and plasma extravasation results from local release of substance P (SP) by antidromic nerve stimulation of sensory nerve endings (*3*). It has been proposed that SP acts as a neurogenic mediator of the inflammatory response (*3*) since it is thought to be a neurotransmitter in sensory afferents (*4*) and since exogenous SP is capable of eliciting responses characteristic of inflammation (*3, 5*). Experiments with capsaicin, the irritating agent of red pepper, support this hypothesis. Capsaicin seems to release SP from sensory afferents, causing permanent damage with concomitant reduction in the SP levels (*6, 7*). Retrobulbar injection of capsaicin initially causes a strong inflammatory response which subsides slowly (*8, 9*); the response to a subsequent trauma, but not that to exogenous SP, is greatly inhibited and this inhibition seems to be long-lasting (*9*). That the response of the uvea to capsaicin depends on an intact trigeminal nerve is suggested by the fact that it is prevented or greatly reduced by denervation (*10*) or prior treatment with tetrodotoxin (*11*). The SP-containing nerve fibers in the anterior uvea are associated with iris smooth muscle and ciliary processes (*12, 13*). Intracameral (into the anterior eye chamber) injection of SP causes miosis and breakdown of the blood-aqueous barrier, thereby mimicking the response to trauma (*5*).

Among a series of SP analogs that has been synthesized (*14, 15*), one, [D-Pro^2, D-Trp^{7,9}]SP, is a weak agonist and a potent, specific, competitive antagonist of SP (*15, 16*). The isolated rabbit iris sphincter pupillae muscle responds to electrical nerve stimulation with a contraction; neither adrenergic nor cholinergic blockade abolishes this response. The SP antagonist, however, prevents the nerve-mediated contraction, suggesting that SP is the neurotransmitter involved (*16*). We have further examined the role of SP in the inflammatory process by analyzing the effects of [D-Pro^2, D-Trp^{7,9}]SP on the response of the rabbit eye to a standardized trauma.

Adult pigmented rabbits (1.5 to 3 kg) of mixed strain were used. They were anesthetized with methohexital sodium (Brietal, Lilly; 5 mg/kg) when given injections into the eyes. No anesthesia was administered during the rest of the experiments. The blood-aqueous barrier of the eye was disrupted by infrared irradiation of the iris for 2 minutes (*17*), or intravitreal injection of SP. The time course of the barrier damage was followed by photoelectric measurement (*18*) of the aqueous flare response (AFR) in the intact eye. This response is a Tyndall phenomenon in the anterior chamber, reflecting protein leakage across the blood-aqueous barrier. A correlation between the AFR and protein concentration has been established (*19*). The results are expressed in arbitrary units with reference to a standard. The AFR and pupillary diameter were measured every 30 minutes (if not otherwise stated). The corneal sensitivity was tested at the same time intervals by the use of wetted cotton swabs. Synthetic SP (Beckman, Geneva, Switzerland) and [D-Pro^2, D-Trp^{7,9}]SP were dissolved in 0.9 percent saline to give stock solutions with concentrations of $4 \times 10^{-5} M$ and $1.7 \times 10^{-3} M$, respectively. The stock solutions of SP and [D-Pro^2, D-Trp^{7,9}]SP were serially diluted so that the volume injected was the same for the different doses (9 μl for SP and 60 μl for the SP analog). The solutions were injected into the corpus vitreum of the left eye unless otherwise stated. The corresponding volume of 0.9 percent saline was injected into the right eye (control eye). Intravitreal injections were given by means of Hamilton precision syringes 3 to 4 mm posterior to the limbus. The SP analog was injected 3 to 4 hours before irradiation of the iris or injection of SP, and the inflammatory response was followed for another 2 to 3 hours. In one series of experiments 60 μl of atropine (Isopto-Atropin, Alcon; 1 percent) was applied topically onto the cornea 1 hour before irradiation of the iris or injection of SP. In a final series of experiments [D-Pro^2, D-Trp^{7,9}]SP was applied topically onto the cornea in a volume of 60 μl, 1 hour

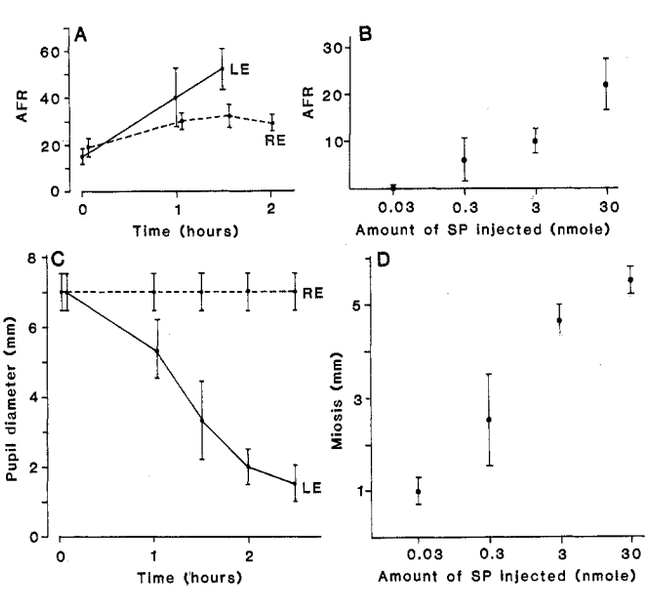

Fig. 1. (A and B) The AFR and (C and D) the miotic response to intravitreal injection of 30 nmole of SP into the left eye (*LE*) and 0.9 percent saline into the right eye (*RE*). The difference between the left and right eye was not significant with respect to AFR and highly significant ($P < .001$) with respect to pupil diameter. (B and D) Dose-response curves showing (B) the maximum AFR (increase over starting value) of the SP-treated eye minus that of the control eye and (D) the decrease in pupil diameter (miosis) (D) after intravitreal injection of SP. The correlation coefficient (r) for AFR versus the amount of SP was .706 ($P < .05$) and for miosis .805 ($P < .01$). Three rabbits in each dose group were examined; vertical lines give the standard error of the mean.

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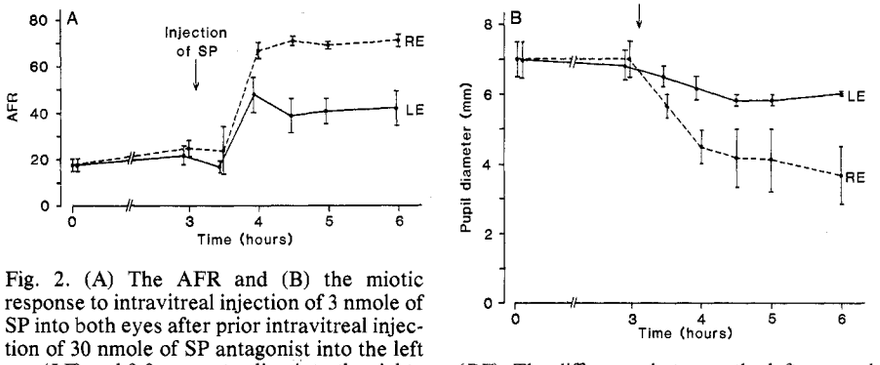
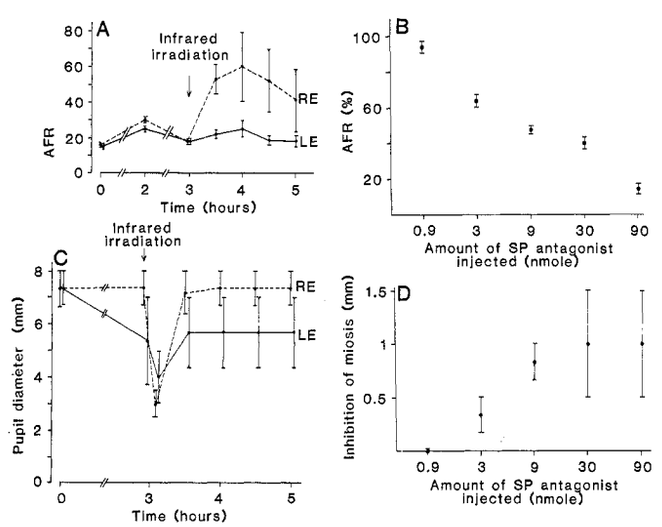


Fig. 2. (A) The AFR and (B) the miotic response to intravitreal injection of 3 nmole of SP into both eyes after prior intravitreal injection of 30 nmole of SP antagonist into the left eye (*LE*) and 0.9 percent saline into the right eye (*RE*). The difference between the left eye and right eye following injection of SP (the 4- to 6-hour interval) was highly significant ($P < .001$) with respect to both AFR and miosis. Three rabbits were tested; vertical lines give the standard error of the mean.

before irradiation of the iris. Statistical analyses were based on Student's *t*-test or regression analysis.

Intravitreal injection of SP in the two highest doses produced strong miosis and a slight AFR. The lower doses of SP produced moderate miosis and no AFR (Fig. 1). Topical application of atropine affected neither the miosis nor the AFR after injection of SP (not shown). Infrared irradiation of the iris caused prompt miosis and a strong AFR. Topical application of atropine before irradiation of the iris did not prevent either the miotic response or the AFR; on the contrary, the AFR was aggravated by approximately 20 percent when atropine was applied ($P < .05$, $N = 5$).

In itself, intravitreal injection of low doses of [D-Pro^2, D-Trp^{7,9}]SP had no effects on the eye. The two highest doses tested gave a moderate, rather long-lasting miosis (Figs. 2 and 3); the corneal sensitivity was not affected. Intravitreal injection of 0.9 percent saline resulted in a small AFR. Injection of the SP analog produced an even smaller AFR than that seen after injection of 0.9 percent saline (Figs. 2 and 3). Prior treatment with [D-Pro^2, D-Trp^{7,9}]SP by intravitreal injection reduced the inflammatory response to SP (Fig. 2). The inflammatory response to infrared irradiation was dose dependently reduced by treatment with [D-Pro^2, D-Trp^{7,9}]SP. The highest dose tested greatly reduced the AFR (by more than 80 percent) and also inhibited miosis (Fig. 3). Combined treatment with atropine and the SP analog had the same effects as treatment with the SP analog alone (not shown). Treatment with 3 nmole of SP did not reduce the effect of subsequent irradiation of the iris (three rabbits) or of another SP injection 3 hours later (three rabbits) (not shown), a finding that seems to exclude the involvement of densitization—that is, the development of tolerance upon repeated administration of a drug. It is interesting that [D-Pro^2, D-Trp^{7,9}]SP could be administered topically to give the same effects on the AFR and miosis as after intravitreal injection, although larger amounts had to be given (Fig. 4).



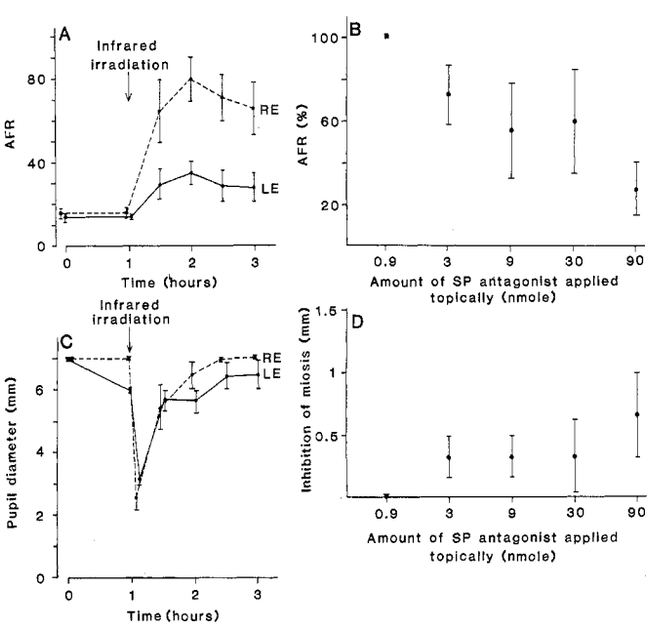


Fig. 3 (left). (A and B) The AFR and (C and D) the miotic response to infrared irradiation of the iris of both eyes after prior intravitreal injection of 90 nmole of [D-Pro^2, D-Trp^{7,9}]SP into the left eye (*LE*) (three rabbits) and 0.9 percent saline into the right eye (*RE*). The difference between the left and right eye after irradiation was highly significant ($P < .001$) with respect to AFR. (B and D) Dose-response curves showing the effect of intravitreally injected SP antagonist on (B) the AFR and (D) the miosis after infrared irradiation of the iris. The AFR in the eye treated with the SP antagonist is expressed as the percentage of the AFR in the control eye at the time of maximum response. Inhibition of miosis is expressed as the difference in pupillary diameter between the eye treated with the SP antagonist and the control eye measured immediately after cessation of irradiation. The correlation coefficient (*r*) for AFR versus the amount of SP analog was .966 ($P < .001$). The regression for inhibition of miosis was not significant, although the overall inhibition was clearly significant ($P < .01$). There were three rabbits in each group; vertical lines give the standard error of the mean. Fig. 4 (right). (A and B) The AFR and (C and D) the miotic response to infrared irradiation of the iris of both eyes after prior topical treatment of the left eye (*LE*) with 90 nmole of SP antagonist (three rabbits) and the right eye (*RE*) with 0.9 percent saline. The difference between the left eye and right eye after irradiation was highly significant ($P < .001$) with respect to AFR. (B and D) Dose-response curves showing the effect of topically administered SP antagonist on (B) the AFR and (D) on the miosis after infrared irradiation of the iris. The AFR in the eye treated with the SP antagonist is expressed as the percentage of the AFR in the control eye at the time of maximum response. Inhibition of miosis is expressed as the difference in pupillary diameter between the eye treated with the SP antagonist and the control eye measured immediately after cessation of irradiation. The regression for AFR versus the amount of SP analog was not significant, although the overall decrease was highly significant ($P < .001$). The regression for inhibition of miosis was not significant but the overall inhibition was significant by $P < .01$. Three rabbits were in each group; vertical lines give the standard error of the mean.

The inflammatory response to trauma in the eye may be mediated by neurogenic factors, conceivably through axon reflexes (*2, 10*). Cholinergic mechanisms do not seem to be involved (*2*). Prostaglandins have been proposed as mediators of inflammatory responses to certain ocular trauma, such as anterior chamber paracentesis and laser irradiation of the iris (*20*). Conceivably also prostaglandins act via neurogenic mechanisms; an intact sensory innervation of the eye is a prerequisite for prostaglandin-evoked responses in the rabbit eye (*21*). Intracameral injection of SP evokes effects similar to those seen after acute trauma to the eye (*5*). Therefore, since SP exists in nerve fibers of the uvea (*12, 13*), SP may be one of the anticipated neurogenic mediators of the vasodilatation, disruption of the blood-aqueous barrier, and miosis associated with inflammation in the eye. Intravitreal application of [D-Pro^2, D-Trp^{7,9}]SP greatly reduced not only the effects of exogenous SP but also the inflammatory response to trauma to the eye. Since [D-Pro^2, D-Trp^{7,9}]SP is a specific SP antagonist (*16*), these observations support the view that SP is one of the neurogenic mediators of the inflammatory response [see (*22*)], and moreover suggest a possible clinical use of SP antagonists in alleviating inflammatory symptoms in the eye, particularly since topical application was sufficient to reduce inflammation. The corneal sensitivity seemed unaffected by treatment with the SP antagonist, suggesting that the sensory afferents in the cornea do not depend upon SP for nociception (*13, 23*).

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Dopamine Receptor Binding Is Increased in Diabetic Rats

Abstract. *The binding of [^{3}H]spiperone, a dopamine receptor ligand, to striatal membranes was increased 30 to 35 percent in rats made diabetic with alloxan or streptozotocin. Binding of [^{3}H]spiperone was normal in rats made diabetic with alloxan but treated with insulin. Thus the number of dopamine receptors and central dopaminergic transmission may be altered in diabetes.*

We previously found that glucose administration rapidly and completely suppresses the firing of dopamine (DA)-containing neurons innervating the rat striatum (*1*). Since DA receptor sensitivity can be increased by treatments that cause sustained reductions in the concentration of intrasynaptic DA (*2*), it is of interest to examine the effects of chronic hyperglycemia on DA receptor sensitivity. We now report the effect of diabetes induced by alloxan or streptozotocin on the binding of [^{3}H]spiperone to membranes prepared from striatal tissues. The in vitro binding of [^{3}H]spiperone appears to provide a reliable index of DA receptor sensitivity (*3*). An examination of DA receptor binding in diabetic animals is also desirable in view of the evidence implicating changes in the function of dopaminergic neurons in the etiology of behavioral and mood disorders (*4*) and emotional disturbances sometimes associated with diabetes (*5*).

Male Sprague-Dawley rats (Zivic-Miller) weighing 200 to 275 g were housed in groups of five to six per cage. They were provided with unrestricted quantities of Purina Rat Chow and tap water and kept in a room with 12-hour cycles of light (600 to 1800 hours) and darkness.

In an initial experiment, the rats were injected subcutaneously with alloxan monohydrate dissolved in 0.9 percent saline (200 mg/kg; $N = 15$) or with 0.9 percent saline (2 ml/kg; $N = 16$). Both groups were decapitated at 1000 to 1400 hours 6 weeks later. Striata were dissected on ice, immediately frozen on dry ice, and stored at −80°C until being analyzed for [^{3}H]spiperone binding. Five pools of striatal tissues, each from two or three diabetic rats, and six pools of tissues, each from two or three controls, were assayed in triplicate with five concentrations of spiperone (0.1 to 2.4 n*M*) (*6*). Nonspecific binding was assessed by assaying a second set of samples in the presence of a saturating concentration (10 μ*M*) of the DA receptor antagonist (+)-butaclamol. Specific binding was defined as the difference in [^{3}H]spiperone binding in the presence and absence of (+)-butaclamol. The maximum specific binding and dissociation constants were calculated from least-squares fits of Scatchard plots of the binding data (*7*). Blood glucose concentrations were also determined for each animal (*8*).

Blood glucose was greatly elevated in all of the alloxan-treated rats (Table 1), indicating the effectiveness of the alloxan treatment (*9*). Maximal spiperone binding was 30 percent greater in the alloxan-treated rats ($P < .01$, Student's *t*-test), but the dissociation constant for spiperone was not altered (Table 1). Thus the number of striatal DA receptors appears to increase in alloxan-treated rats (*10*).

In a second experiment, diabetes was produced by administering streptozotocin (*11*). Six rats received intraperitoneal injections of streptozotocin (75 mg/kg) and six received saline (2 ml/kg). All the

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