Long-term administration of a substance P antagonist, (D-Pro^2, D-Trp^{7,9})-SP, abolishes the response to ocular trauma

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Summary. The long-term effect of ocular administration of a substance P (SP) antagonist, (D-Pro^2, D-Trp^{7,9})-SP, was studied in the rabbit. After 2–3 months of topical administration of the antagonist twice daily, a mild trauma was applied in the form of infrared irradiation of the iris. The control eye responded with disruption of the blood-aqueous barrier, the eye treated with the antagonist did not. Two days after termination of treatment, the response to ocular injury was still reduced. Another 2 days later, ocular injury evoked a normal response, which shows that the protection was reversible. No adverse reaction to the SP antagonist was noted.

A substance P antagonist was recently shown to inhibit the ocular response to infrared irradiation of the iris[1]. Substance P (SP) has been proposed to be a neurogenic mediator of the ocular response to trauma, since it evokes symptoms of ocular injury[1–3] and since it is released into the aqueous humor of the rabbit eye after stimulation of the trigeminal nerve[2]. The present study deals with the effects of long-term administration of an SP antagonist, (D-Pro^2, D-Trp^{7,9})-SP, onto the eye.

Materials and methods. Drug. The SP analogue was synthetized by techniques analogous to those described elsewhere[4,5]. Its purity was tested by high performance liquid chromatography and found to be better than 98%. It has previously been described as a competitive and fairly specific SP antagonist[6,7]. It was dissolved in sterile 0.9% saline. Solutions were prepared fresh every week and stored in a refrigerator.

Single dose treatment. 6 pigmented rabbits received a single dose of 300 nmoles (50 µl) of (D-Pro^2, D-Trp^{7,9})-SP topically onto the left eye. The right eye served as control and received a single dose of 0.9% saline topically.

Multiple dose treatment. 3 pigmented rabbits received (D-Pro^2, D-Trp^{7,9})-SP topically for 2 months onto the left eye in a dose of 300 nmoles (50 µl) twice daily, at 08.00–10.00 h and 15.00–

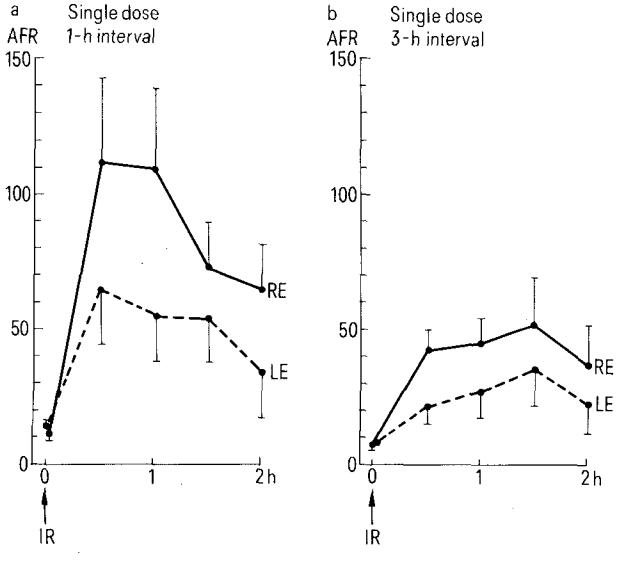

Figure 1. Aqueous flare response (AFR) (mean ± SE) to infrared irradiation (IR) of the iris in the left eye (LE) and the right eye (RE). *a* The left eye of 3 rabbits had been pretreated with a single dose of 300 nmoles SP antagonist 1 h before IR of the iris. The difference between the eyes is highly significant ($p < 0.001$, analysis of variance, 2-way). *b* The left eye of 3 rabbits had been pretreated with a single dose of 300 nmoles SP antagonist 3 h before IR of the iris. The difference between the eyes is highly significant ($p < 0.001$, analysis of variance, 2-way).

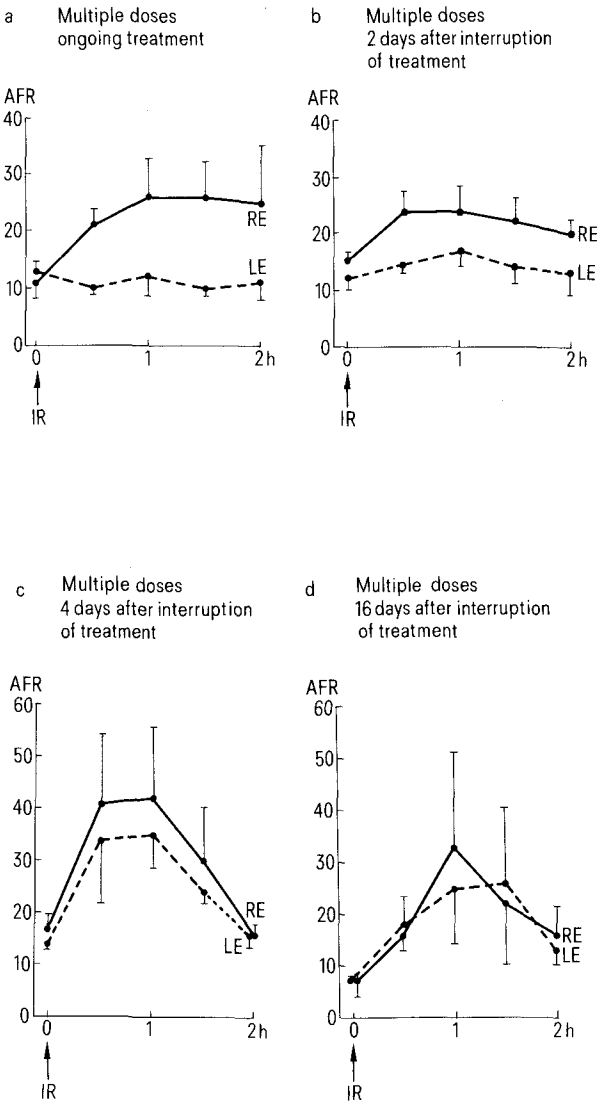

Figure 2. Aqueous flare response (AFR) (mean ± SE) to infrared irradiation (IR) of the iris in the left eye (LE) and the right eye (RE). The left eye of 3 rabbits had been treated with 300 nmoles SP antagonist applied topically twice daily for 2 months. *a* During ongoing treatment 2 months after the start; the difference between the eyes is highly significant ($p < 0.001$, analysis of variance, 2-way). *b* 2 days after termination of treatment; the difference between the eyes is highly significant ($p < 0.001$, analysis of variance, 2-way). *c* 4 days after termination of treatment no significant difference between the eyes ($p > 0.05$, analysis of variance, 2-way). *d* 16 days after termination of treatment; no significant difference between the eyes ($p > 0.05$, analysis of variance, 2-way).

19.00 h, respectively. Another group of 8 pigmented rabbits received (D-Pro^2, D-Trp^{7,9})-SP, topically for 3 months onto the left eye twice daily, at 07.00–09.00 h and 15.00–19.00 h, respectively. 3 of the latter rabbits received 300 nmoles (50 µl) and five 30 nmoles (50 µl). The right eye (control) received 0.9% saline twice a day.

Disruption of the blood-aqueous barrier. The blood-aqueous barrier was disrupted by infrared irradiation (IR)[8] of the iris of both eyes for 2 min. IR is not painful and the animals were conscious but gently restrained. The course of the barrier damage was followed by photo-electric measurements[9] of the aqueous flare response (AFR) every 30 min. This response is a Tyndall phenomenon in the anterior chamber, reflecting protein leakage across the blood-aqueous barrier. A correlation between the AFR and the protein concentration has been established[10]. The method has the advantage of being atraumatic, permitting recording of the AFR at intervals during the experiment. Furthermore, the method detects changes in AFR that cannot be observed by conventional focal illumination. The results are expressed in arbitrary units with reference to a standard[10].

Results. Single dose treatment. 3 rabbits received a single dose of 300 nmoles SP antagonist topically onto the left eye 1 hour before IR of the iris of both eyes. The AFR was greatly reduced in the left eye (fig. 1A). Another group of 3 rabbits received a single dose of 300 nmoles topically onto the left eye 3 h before IR of the iris of both eyes. There was still an inhibition of the AFR in the left eye (fig. 1B). On an another occasion the same 3 rabbits received a single dose of 300 nmoles 5 h before IR of the iris of both eyes. The AFR was no longer inhibited (not shown in fig.).

Multiple dose treatment. At the 2–3 month stage and during ongoing treatment with the SP antagonist the following experiments were performed. 3 animals, which had received the antagonist (300 nmoles) for 2 months, were subjected to IR of the iris of both eyes 1 h after administration of the antagonist. The AFR was abolished in the pre-treated eye (fig. 2A). 6 days later, when the antagonist had been withdrawn for 2 days, the AFR to IR of the iris was still reduced in the pre-treated eye (fig. 2B). Another 2 and 14 days later, respectively, the 3 animals were again subjected to IR of the iris. There was no longer any inhibition of the AFR (fig. 2C, D). Another group of 3 rabbits, pretreated with 300 nmoles SP antagonist for 3 months, was subjected to IR of the iris of both eyes about 30 min after application of the SP antagonist. The AFR was greatly inhibited (fig. 3A). IR of the iris was also performed on the 5 rabbits treated with 30 nmoles of the SP antagonist for 3 months. The AFR was greatly reduced (fig. 3B). During and after long-term treatment with the SP antagonist no adverse reactions were observed.

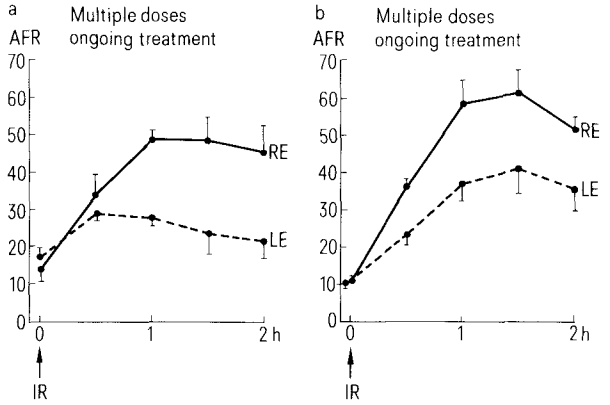

Figure 3. Aqueous flare response (AFR) (mean ± SE) to infrared irradiation (IR) of the iris in the left eye (LE) and the right eye (RE). *a* The left eye of 3 rabbits was pretreated with 300 nmoles SP antagonist applied topically twice daily for 3 months. The right eye was pretreated with saline. The difference between the eyes is highly significant ($p < 0.001$, analysis of variance, 2-way). *b* The left eye was pretreated with 30 nmoles SP antagonist for 3 months and the right eye with saline ($n = 5$). The difference between the eyes is highly significant ($p < 0.001$, analysis of variance, 2-way).

Discussion. The responses to ocular injury are thought to depend at least partially upon agents released from peripheral sensory nerve fibers[1, 11–13]. SP has been proposed as a candidate mediator for the neurogenic response because 1) exogenous SP evokes symptoms of ocular injury[1–3, 12], 2) SP occurs in trigeminal nerve fibers[2, 14–17], and 3) specific antagonists to SP suppress the responses not only to SP[1] but also to IR[1], bradykinin[11], prostaglandin[18] and capsaicin[18].

The present work confirms and extends the previous finding that topical application of an SP antagonist inhibits the response to IR of the iris[1]. The inhibitory effect of a single topical administration of the antagonist persisted for 3 h. Long-term treatment with the SP antagonist abolished the response to IR of the iris. The response to ocular trauma was reduced 2 days after termination of treatment, suggesting that with time the antagonist accumulates in the eye. Another 2 days later IR again produced AFR. Thus, the effect was reversible. Furthermore, long-term treatment with the SP antagonist did not reveal any adverse reactions during or after 3 months of treatment with very large doses[19].

1 Holmdahl, G., Håkanson, R., Leander, S., Rosell, S., Folkers, K., and Sundler, F., Science *214* (1981) 1029.
2 Bill, A., Stjernschantz, J., Mandahl, A., Brodin, E., and Nilsson, G., Acta physiol. scand. *106* (1979) 371.
3 Stjernschantz, J., Sears, M., and Stjernschantz, L., Invest. Ophthal. vis. Sci. *20* (1981) 53.
4 Folkers, K., Hörig, J., Rosell, S., and Björkroth, U., Acta physiol. scand. *111* (1981) 505.
5 Rosell, S., Björkroth, U., Hörig, J., Xu, J.-C., and Folkers, K., Eighth Intern. Congr. Pharm. Tokyo, Japan, Abs., in press (1983).
6 Leander, S., Håkanson, R., Rosell, S., Folkers, K., Sundler, F., and Tornqvist, K., Nature, Lond. *294* (1981) 467.
7 Folkers, K., Håkanson, R., Hörig, J., and Leander, S., Br. J. Pharmac. (1984) in press.
8 Dyster-Aas, K., and Krakau, C.E.T., Invest. Ophthal. *3* (1964) 127.
9 Bengtsson, E., Krakau, C.E.T., and Öhman, R., Invest. Ophthal. *14* (1975) 306.
10 Anjou, C.I.N., and Krakau, C.E.T., Acta ophthal. *39* (1961) 1.
11 Bynke, G., Håkanson, R., Hörig, J., and Leander, S., Eur. J. Pharmac. *91* (1983) 469.
12 Butler, J.M., and Hammond, B.R., Br. J. Pharmac. *69* (1980) 495.
13 Butler, J.M., Unger, W.G., and Cole, D.F., Q. Jl exp. Physiol. *65* (1980) 261.
14 Olgart, L., Gazelius, B., Brodin, E., and Nilsson, G., Acta physiol. scand. *101* (1977) 510.
15 Brodin, E., Gazelius, B., Lundberg, J.M., and Olgart, L., Acta physiol. scand. *111* (1981) 501.
16 Tervo, K., Tervo, T., Eränkö, L., Eränkö, O., and Cuello, A.C., Histochem. J. *13* (1981) 435.
17 Tornqvist, K., Mandahl, A., Leander, S., Lorén, I., Håkanson, R., and Sundler, F., Cell Tiss. Res. *222* (1982) 467.
18 Bynke, G., Håkanson, R., and Hörig, J., Experientia *39* (1983) 996.
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