Substance P-immunoreactive nerve fibres in the anterior segment of the rabbit eye

Distribution and possible physiological significance

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Summary. Substance P-immunoreactive nerve terminals were found in several locations in the anterior segment of the rabbit eye. In the iris they occurred in the sphincter muscle and were randomly distributed in the iris stroma with some fibres running close to the dilator muscle. In the ciliary body these immunoreactive elements were few and occurred within bundles of nerve fibres, while in the ciliary processes they were more numerous with a predominantly subepithelial location. Blood vessels in the anterior uvea were often surrounded by substance P-immunoreactive fibres. No substance P-fibres were found in the cornea, while the sclera contained very few such elements.

Using conventional in vitro techniques it was found that the sphincter pupillae muscle of the iris responded to electrical stimulation with a contraction that was resistant to cholinergic and adrenergic blockade, but was inhibited by the neuronal blocker tetrodotoxin. This indicates the existence of a non-cholinergic, non-adrenergic neuronal mediator of the contractile response. Exogenously applied substance P produced a long-lasting contraction of the sphincter muscle, an observation compatible with the view that substance P is the noncholinergic, non-adrenergic neurotransmitter involved.

Key words: Neuropeptides – Substance P – Uvea – Immunocytochemistry – Iris – Smooth muscle contraction – Rabbit

Substance P (SP) is a neuropeptide with a widespread distribution in the central nervous system (e.g., Hökfelt et al. 1977; Ljungdahl et al. 1978), including the retina (Karten and Brecha 1980), and in the peripheral nervous system (e.g., Nilsson et al. 1975; Hökfelt et al. 1977; Alm et al. 1978). SP occurs in primary sensory neurones (Hökfelt et al. 1975a, b), and it has been suggested that at least a proportion of the peripheral SP-containing fibres represent the dendritic ramifications of such neurones. It has also been suggested that SP is a causative factor in the “irritative”
response to antidromic nerve stimulation and consequently involved in “axon reflexes” (Lembeck and Holzer 1979). This hypothesis is supported by the finding that SP-immunoreactive material is released from the tooth pulp upon electrical stimulation of the mandibular nerve (Olgart et al. 1977). The presence of SP in the anterior uvea of the rabbit is suggested by the results of radioimmunoassay (Butler et al. 1980; Camras and Bito 1980). Electrical or mechanical stimulation of the trigeminal nerve causes an increase in the amount of SP-like material in the aqueous humour, concomitant with miosis, vasodilation, breakdown of the blood-aqueous barrier and a rise in the intraocular pressure (Bill et al. 1979). In the same species intracameral injection of SP mimics these effects (Bill et al. 1979). Surgical denervation as well as treatment with capsaicin markedly reduces the SP concentration in the anterior uvea and modifies the reaction to irritative substances such as prostaglandin E₁, nitrogen mustard, bradykinin and capsaicin, but not the reaction to SP (Butler and Hammond 1980; Camras and Bito 1980). Together these results suggest that SP is one of the substances released by antidromic nerve stimulation to act as a mediator of irritative responses.

The present report deals with the distribution of SP-immunoreactive nerve fibres in the anterior segment of the rabbit eye and the possible functional significance of SP fibres in the iris.

Materials and methods

Immunocytochemistry

Eight albino or pigmented rabbits weighing 1–2 kg were used. Six rabbits were anaesthetized with Brietal® and perfused through the ascending aorta with 1 l of an ice-cold 4% formalin solution (40 g paraformaldehyde in 110.1 M phosphate buffer; pH adjusted to 7.0 with 1 N NaOH). The anterior uvea was rapidly dissected out and stored in the fixative for 24–48 h and rinsed for 24 h in ice-cold Tyrode buffer containing 5% sucrose. The specimens were frozen on dry ice and sectioned in a cryostat at −15°C. Two rabbits were killed by i.v. injection of air. The irides were rapidly dissected out and placed as whole mounts on glass slides. The pigment layer was removed by gentle scraping with a scalpel. The preparations were fixed by immersion in buffered picric acid-formaldehyde solution overnight. They were then dehydrated in graded ethanol solutions, cleared in xylene and rehydrated (cf. Furness et al. 1980). The cryostat sections (15 µm) and whole-mount preparations were processed for the immunocytochemical demonstration of SP using the indirect immunofluorescence technique (Coons et al. 1955). Each section was exposed to SP antiserum in the appropriate dilution for 3 h at room temperature in a moisture chamber. After thorough rinsing in phosphate-buffered saline, pH 7.2, the site of the antigen-antibody reaction was revealed with fluorescein isothiocyanate labelled sheep anti-rabbit Ig G (SBL, Stockholm, Sweden), used in dilution 1:20. Incubation was for 30 min at room temperature. The antisera contained 0.25% serum albumin and 0.25% Triton X-100. For details of the SP antisera used, see Table 1. Immunofluorescence was observed in a fluorescence microscope with filters selected to give peak excitation at 490 nm. Controls were as follows: 1) the SP antiserum was omitted; 2) the second (fluoresceinated) antibody was omitted; 3) normal rabbit antiserum was applied instead of SP antiserum; 4) each SP antiserum was permitted to react with synthetic SP (10–100 µg/ml dilute antiserum) for at least 24 h before being applied to the sections. Such absorption of the antisera resulted in abolishment of the immunoreaction.

For reasons of convenience and simplicity we refer to the immunoreactive material as SP although it must be realized that the antibodies will detect also other peptides or proteins that share the immunogenic amino-acid sequence with SP (see Table 1).

Motor activity studies

The functional properties of the isolated iris sphincter pupillae muscle were studied using conventional in vitro techniques. The muscles were excised from 12 pigmented rabbits (1–2 kg), opened and mounted.