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A dual action of 5-hydroxytryptamine on the ovarian suspensory ligament of the rat

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5-Hydroxytryptamine has a dual effect on the spontaneously contracting rat ovarian ligament, *in vitro*, a contraction which is antagonized by the prior administration of methysergide and a relaxation of the ligament observed in the methysergide-treated preparation. The relaxatory effect was not antagonized by propranolol or tetrodotoxin but treatment of the ligament with indomethacin abolished this response. Prostaglandins of the E series produced an inhibition, and $PGF_{2\alpha}$ a contraction of the ligament. Thin layer chromatographic separation indicates that 5-HT causes the release of a PGE_2-like substance which relaxes the ovarian suspensory ligament.

The ligamentum suspensorium ovarii (ovarian suspensory ligament) is a fine structure of smooth muscle which attaches the anterior end of the uterus and associated ovary to the dorsal wall of the abdominal cavity adjacent to the diaphragm (Drahn, 1924). This ligament exhibits regular spontaneous activity *in vitro* (Melton & Saldivar, 1970).

MATERIALS AND METHODS

The ovarian suspensory ligament preparation

Mature virgin female Wistar rats (210–280 g) were killed and the abdomen opened. The ovarian suspensory ligament was cut free after placing ties adjacent to the ovary and to the diaphragm. It was placed in Krebs solution and carefully dissected free of surrounding fatty tissues, and suspended in an organ bath (10 ml), containing modified Krebs bicarbonate solution of composition: NaCl 7·00, KCl 0·35, $NaHCO_3$ 2·10, $MgSO_4 \cdot 7H_2O$ 0·14, KH_2PO_4 0·16, $CaCl_2 \cdot 2H_2O$ 0·29, dextrose 2·00 g litre^{-1} of distilled water gassed with 5·0% CO_2 in oxygen at 37°. Changes in tension produced by the ligament were detected by a Devices force transducer and the records were displayed on a Devices M2 heat pen recorder.

Collection of samples to determine prostaglandin-like activity

Samples of bathing fluid were siphoned off from 6 ligaments and replaced every 15 min. Four samples were collected for the control period in the absence of 5-HT; during increased ligament contractility produced by 5-HT (0·2 μg ml^{-1}); during relaxation of the ligaments produced by 5-HT (0·2 μg ml^{-1}) after pretreatment with methysergide (0·2 μg ml^{-1}) for 15 min; and following the addition of 5-HT (0·2 μg ml^{-1}) to the bathing medium of ligaments treated with methysergide (0·2 μg ml^{-1}) for 15 min and indomethacin (1 μg ml^{-1}) for 30 min. The group samples were pooled and the prostaglandins were extracted from the aqueous phase for chromatographic separation by acidification of the aqueous phase to pH 3·5 with 1·0 N HCl and extracting twice with equal volumes of ethyl acetate (Gilmore, Vane & Wyllie, 1968). The ethyl acetate was evaporated to dryness under reduced pressure at 50° and the residue was stored at −12° until required for chromatographic separation.

Thin layer chromatography

The AII system described by Greén & Samuelsson (1964) was used to determine which of the prostaglandins of the E series (PGE) the active principle most resembled. Detection of the prostaglandins by spraying the dried plates with 10% phosphomolybdic acid in ethanol followed by heating at 85° for 15 min as described by Greén & Samuelsson (1964) was only successful for the reference prostaglandins, the amounts present in the samples being too small to detect by this method. Instead, the reference prostaglandins were spotted on the left and the extracts on the right of the plate, and following the separation procedure the reference prostaglandins were identified and the other half of the plate was divided into regions. The silica gel was removed and extracted into 2 ml methanol. After centrifuging at 1000 rev min^{-1} for 10 min the residue was washed with 2 ml methanol and centrifuged. The supernatants were dried and dissolved in 1 ml Krebs bicarbonate solution.

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Prostaglandin determination

The samples were tested for prostaglandin-like activity using the superfusion technique of Gilmore & others (1968) and Vane (1971) on the rat stomach strip. To increase the specificity of the method, the Krebs bicarbonate solution contained hyoscine, mepyramine and methysergide (0·1 μg ml^{-1} each).

The drugs used were: 5-hydroxytryptamine creatinine sulphate (5-HT) (Koch Light), methysergide bimaleate (Sandoz), tetrodotoxin (Calbiochem), indomethacin (Merck, Sharp & Dohme) (−)-hyoscine hydrobromide, mepyramine maleate (May & Baker), prostaglandins E_1 E_2 and $F_{2\alpha}$ (Upjohn).

RESULTS

Ovarian suspensory ligament

The dual effect of 5-HT on the ovarian suspensory ligament is shown in Fig. 1. The ovarian suspensory ligament exhibited regular spontaneous contractile activity, 5-HT (0·2 μg ml^{-1}) produced a sustained contraction which was antagonized by methysergide (0·2μg ml^{-1}) added to the bathing fluid 15 min before the agonist. In the presence of methysergide, 5-HT inhibited spontaneous activity and relaxed the ligament by a mechanism unaffected by propranolol (0·4 μg ml^{-1}) or tetrodotoxin (0·05 μg ml^{-1}). However, indomethacin (1 μg ml^{-1}) in the bathing medium for 30 min prevented the inhibitory action of 5-HT on the methysergide-treated ovarian suspensory ligament. Prostaglandins E_1 and E_2 (0·01 μg ml^{-1}) produced an inhibition of spontaneous activity similar to that seen with 5-HT in the methysergide-treated preparation; and prostaglandin $F_{2\alpha}$ (0·02 μg ml^{-1}) produced a contraction (Fig. 1.)

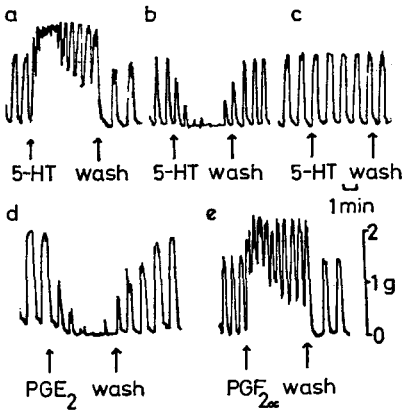

FIG. 1. Records of the spontaneously active rat ovarian suspensory ligament showing (a) the effects of 5-HT (0·2 μg ml^{-1}); (b) the effects of 5-HT (0·2 μg ml^{-1}) after methysergide (0·2 μg ml^{-1}), and (c) blockade by indomethacin (1 μg ml^{-1}) of the inhibitions induced by 5-HT (0·2 μg ml^{-1}). Record (d) shows the effect of PGE_2 (0·01 μg ml^{-1}) and (e) the effect of $PGF_{2\alpha}$ (0·02 μg ml^{-1}).

Identification of prostaglandin-like activity

Extracts of the samples of Krebs bicarbonate solution were found to contain prostaglandin-like activity. Thin layer chromatographic separation in the AII solvent system showed the presence of active material with an R_F value similar to that of PGE_2 (0·70) but no activity on the plate corresponding to the R_F value of PGE_1 (0·86). Total activity assayed on the rat stomach strip was not confined to the region on the plate corresponding to the R_F of PGE_2 but was also found in the region adjacent to the PGE_2 zone. For example, in one experiment, total activity from extracts taken during 5-HT (0·2 μg ml^{-1}) induced contraction of the ligaments was found to be in the proportions of 92·5% in the plate region of R_F 0·70 and 7·5% in the region of R_F 0·63.

In 5 experiments, extracts taken during the control periods of normal spontaneous activity were found to contain 1·3 ± 0·3 μg g^{-1} min^{-1} PGE_2-like activity. 5·2 ± 0·4 μg g^{-1} min^{-1} PGE_2-like activity was found in extracts taken during 5-HT (0·2 μg ml^{-1}) produced contraction of the ovarian suspensory ligaments and 4·7 ± 0·2 μg g^{-1} min^{-1} PGE_2-like activity found in extracts taken during 5-HT produced relaxation of the methysergide treated ligaments. No PGE_2-like activity was detected in extracts taken during the period that 5-HT was without effect on the methysergide (0·2 μg ml^{-1}) and indomethacin (1 μg ml^{-1}) treated ligaments.

DISCUSSION

5-HT exerted a dual action on the rat ovarian suspensory ligament; a contraction mediated through receptors which are blocked by methysergide and an inhibition in the methysergide-treated preparation. 5-HT has been shown to produce indirect β-sympathomimetic effects on the rabbit heart (Fozard & Mwaluko, 1975) and guinea-pig trachea (Coleman & Levy, 1974), but the inhibitory effect of 5-HT on the rat ovarian suspensory ligament was not antagonized by propranolol and therefore not mediated through β-adrenoceptors. Gershon (1967) showed that the inhibitory activity of 5-HT on some intestinal smooth muscle preparations was antagonized by tetrodotoxin which selectively blocks neuronally elicited responses, (Kao, 1966; Ogura, Mori & Watanabe, 1966). Tetrodotoxin did not modify the inhibitory effect of 5-HT on the methysergide-

treated ligament, nor did it alter the spontaneous activity or contraction produced by 5-HT indicating that none of these effects are mediated through reflex nerve stimulation.

The involvement of prostaglandin-like activity was indicated when indomethacin, a potent inhibitor of prostaglandin biosynthesis (Ferriera, Moncada & Vane, 1971), prevented the inhibitory effect of 5-HT and at the same time was shown to inhibit the release of prostaglandin-like material to below detectable concentrations. The prostaglandins of the E series but not $F_{2\alpha}$ produced a similar relaxation of the ligament and the chromatographic separation of extracts and subsequent biological determination indicate that 5-HT increases the rate of release of PGE_2-like material from the rat ovarian suspensory ligament.

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