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17. We thank Maurice Rosenstraus for valuable assistance. Supported by grants V117A and V117B from the American Cancer Society.

11 November 1974

Prostaglandins in Human Seminal Fluid: Two Novel Compounds

Abstract. *Human seminal fluid frozen immediately after ejaculation contains two novel prostaglandins. These are present in larger quantities than the previously reported prostaglandins. They are characterized by gas chromatography and mass spectrometry as 19-hydroxyprostaglandins E_1 and E_2. Most of the previously identified prostaglandins may be artifacts.*

The prostaglandin content of human seminal fluid that had been frozen immediately after ejaculation was found to differ significantly from that which had not been so treated (*1*). The initial studies of prostaglandins were performed on pooled samples of human seminal fluid obtained from fertility investigation laboratories (*2*). Thirteen prostaglandins were identified: A_1, A_2, B_1, B_2, E_1, E_2, E_3, $F_{1\alpha}$, $F_{2\alpha}$, and 19-hydroxy A_1, A_2, B_1, and B_2. The 19-hydroxy compounds were the most abundant, their concentrations being approximately four times those of prostaglandins of the E series.

Human seminal fluid was obtained from a healthy fertile donor on two separate occasions and cooled to −10°C within 5 minutes. An extract in a mixture of chloroform and methanol (2 : 1) was evaporated under nitrogen and remaining traces of water were removed by dissolving the residue in benzene and evaporating to dryness (*3*). On silicic acid chromatography, "neutral lipid," "prostaglandin," and "polar lipid" fractions were eluted, respectively, by chloroform, a mixture of chloroform and methanol (8 : 1), and methanol. The "prostaglandin" fraction was examined by gas chromatography–mass spectrometry (GC-MS) without further purification (*4*).

The following derivatives of the prostaglandin fraction were prepared for GC-MS analyses (*5*): the *O*-ethyloxime, trimethylsilyl ester, trimethylsilyl ether (EO-TMS); the *O*-methyloxime (MO)-TMS; and the MO-[9-^{2}H]TMS derivatives. Selective ion monitoring (*6*) at a mass-to-charge ratio (*m/e*) of 611 during GC-MS of EO-TMS derivatives revealed the presence of prostaglandins E_1 and E_2 as well as more abundant components of longer retention time. The GC data (Table 1) indicated that the additional components (compounds **1** and **2**) were 19-hydroxyprostaglandins E_1 and E_2 (*7*). Comparison of the mass spectral data (Table 2) with those of the primary prostaglandin derivatives (*8*) confirmed that the additional hydroxyl groups were at the 19-position. Ions in the spectra of derivatives of prostaglandins E_1 and E_2 which do not contain the C_{16} to C_{20} chain are present in spectra of derivatives of **2** and **1**, respectively. Ions characteristic of the unsubstituted C_{16} to C_{20} chain are absent. Instead, corresponding ions containing the trimethylsilyloxy group appear at 88 mass units higher. Additional ions are formed by elimination of trimethylsilanol from these ions. The powerful fragmentation-directing properties of the 19-trimethylsilyloxy groups give rise to characteristic ions of *m/e* 129 and 143 comprising C_{17} to C_{19} and C_{17} to C_{20}, respectively.

Thus, all the data are consistent with compounds **1** and **2** being 19-hydroxy prostaglandins E_2 and E_1, respectively. Since meticulous precautions were taken to minimize the possibility of modifications of the samples during storage, isolation, and characterization, it is reasonable to assume that 19-hydroxy prostaglandins E_1 and E_2 are indeed present in human seminal fluid immediately after ejaculation.

Prostaglandins A_1, A_2, B_1, B_2, and their 19-hydroxy analogs were not found in our study. Since others have found them in seminal fluids, that had not been frozen immediately after collection, the possibility exists that they are metabolites produced after ejaculation. However, since prostaglandins of the E series are readily dehydrated to those of the A and B series by heat, light, and extremes of *p*H (*9*), it seems more likely that some, if not all, of the prostaglandins of the A and B series (and their 19-hydroxy analogs) previously found in human seminal fluid are artifacts.

19-Hydroxy prostaglandins E_1 and E_2 were found in approximately five times the concentration of prostaglan-

Table 1. Gas chromatographic data for derivatives of compounds **1** and **2**. Expected values are computed by adding the appropriate retention increments (+230 for 19-hydroxy, +310 for 20-hydroxy) to retention indices of prostaglandins E_2 and E_1. Each compound affords both a *syn* and an *anti* isomer, which are separated by gas chromatography. Abbreviations: 19-hydroxy, 19-OH; 20-hydroxy, 20-OH.

Derivatives	Compound **1**	Compound **2**
	Observed	
MO-TMS	3040, 3090	3070, 3120
EO-TMS	3095, 3130	3105, 3145
	Expected	
19-OH MO-TMS	3060, 3110	3090, 3140
19-OH EO-TMS	3090, 3145	3120, 3175
20-OH MO-TMS	3140, 3190	3170, 3220
20-OH EO-TMS	3170, 3225	3200, 3255

Table 2. Mass spectral data for isomers of the MO-TMS derivative of compound **1**. Parentheses indicate relative abundances.

Peak	M$^+$	Base peak	Major ions					
Peak 1	685	75	143	117	73	217	133	129
	(2)		(55)	(45)	(45)	(44)	(43)	(38)
Peak 2	685	143	75	133	223	353	129	73
	(3)		(87)	(78)	(73)	(64)	(63)	(43)

dins of the E series. These novel compounds, therefore, are apparently the most abundant prostaglandins in the richest known source of mammalian prostaglandins.

Since this work was completed, the occurrence of 19-hydroxy prostaglandins E_1 and E_2 has been found by Taylor and Kelley (*10*), who also obtained the dehydration products (*11*).

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3. To minimize the possibility of oxidation of the prostaglandins, solvents were freshly distilled and purged with dry nitrogen. All apparatus was flushed with dry nitrogen.
4. Parallel studies with [^{3}H]prostaglandins B_1, E_1, and $F_{1\alpha}$ showed that these compounds were all eluted in the "prostaglandin" fraction.
5. An LKB 9000 instrument, equipped with a glass column (2.7 m by 0.6 cm) containing 1 percent SE-30 on Gas-Chrom Q (100 to 120 mesh); flash heater, 270°C; the column temperature was programmed from 140° to 290°C at 2 degrees per minute; molecular separator, 250°C; electron energy, 22.5 ev.
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11 November 1974

Localized Desensitization of Limulus Photoreceptors Produced by Light or Intracellular Calcium Ion Injection

Abstract. *Spots of light were used to measure the light sensitivity of spatially separated regions of single* Limulus *photoreceptors. The desensitization caused by irradiating part of the cell was largest in the irradiated region. The desensitization caused by intracellular calcium ion injection was largest near the injection site. The spread of desensitization away from the injection site suggests that calcium ion can diffuse over neuronal dimensions, but that the effective rate of diffusion is not so high as to abolish calcium gradients. The results are compatible with the previously proposed hypothesis that a rise in the intracellular calcium ion concentration mediates light adaptation.*

Photoreceptors have the ability to adapt to light and dark, that is, to change their sensitivity. Some photoreceptors are sufficiently large so that small areas of the transducing membrane can be illuminated. The adaptation caused by irradiating part of a cell tends to be localized to the region of illumination (*1*).

Lisman and Brown (*2*) showed that injection of Ca^{2+} reduced the response of *Limulus* ventral photoreceptors to spatially uniform illumination and proposed that a light-induced increase in the intracellular calcium concentration (Ca_i^{2+}) (*3*) is a factor controlling light adaptation. Experiments by Fein (*4*) indicate that in these cells light adaptation is localized to the region of illumination. Therefore, if the Ca^{2+} hypothesis is correct, local changes in sensitivity would be caused by local changes in Ca_i^{2+}. This would imply that cytoplasmic Ca^{2+} gradients can occur over neuronal dimensions (< 100 μm); however, such gradients have not previously been described.

We examined the possibility of cytoplasmic Ca^{2+} gradients by injecting Ca^{2+} into one end of a *Limulus* ventral photoreceptor (schematized in Fig. 1) and monitoring the change in the responses to two spots of light, one placed at the injection site (*A*) and the other at the opposite end of the cell (*B*). The photoreceptors are located on the lateral olfactory nerve, which was dissected, desheathed, and mounted in a small chamber with a transparent base. Single photoreceptors (~ 50 by 150 μm in cross section) (*5*) were observed through a compound microscope ($\times 400$). The cell was illuminated from below by two spots of light that could be positioned on it. All experiments were carried out under visual control. Cells were penetrated with a Ca^{2+}-containing electrode (*6*), which was used both for iontophoretic injection of Ca^{2+} and for recording the responses evoked by the two spots. Despite efforts to minimize light scatter (*7*), the spots, which were nominally 10 μm in diameter, appeared considera-

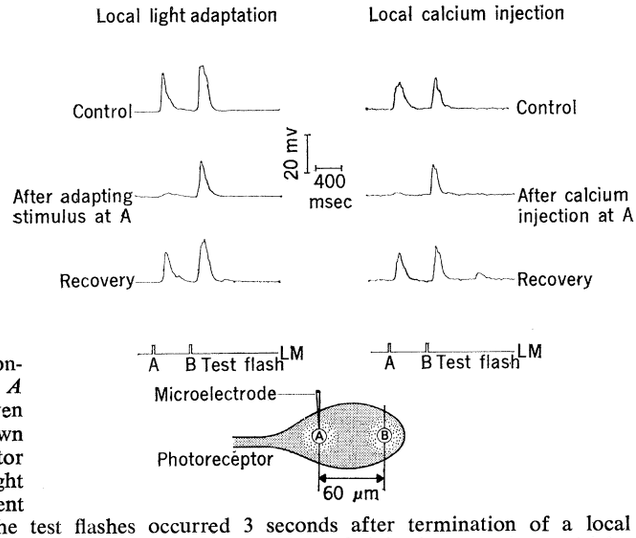

Fig. 1. Localized desensitization produced by a local adapting light or Ca^{2+} injection. The lower part of the figure is a schematized version of the photoreceptor, showing the two stimulus spots labeled *A* and *B*, with the intracellular Ca^{2+}-containing electrode aligned with the spot at *A*. Two test flashes of constant intensity, one at *A* and one at *B*, were given in succession, as shown by the light monitor (*LM*). In the local light adaptation experiment (left, middle trace) the test flashes occurred 3 seconds after termination of a local adapting stimulus at *A* (5 seconds in duration). In the Ca^{2+} injection experiment (right, middle trace), the test flashes occurred 3 seconds after termination of the injections (2 na for 10 seconds). Note that there was a small change in the amplitude of the photoresponse between the local light adaptation and the Ca^{2+} injection experiments.