Effects of adrenergic and cholinergic agonists on adenylate and guanylate cyclase activity of isolated guinea-pig seminal vesicle epithelium[1]

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Summary. Isolated seminal vesicle epithelium of the guinea-pig contained increased amounts of cAMP and cGMP after treatment with PGE_1 and carbachol, respectively. Adrenergic agents had no influence. Possible physiological implications of these results are discussed.

Male accessory sex organs secrete most of the seminal plasma found in the ejaculate[2]. This secretion is completely dependent upon the presence of androgens[3]. Neuronal stimuli may, however, modulate the secretory rate in some of these organs[4,5], as they do in many exocrine glands[6]. The seminal vesicle of the guinea-pig contains the necessary cholinergic and adrenergic nerve fibres[7,8]. One prerequisite for these fibres to influence the secretory rate of the epithelium is the presence of nucleotide cyclases sensitive to the appropriate drugs[9,10]. In this report we describe experiments which probe for the presence of such cyclases in vitro.

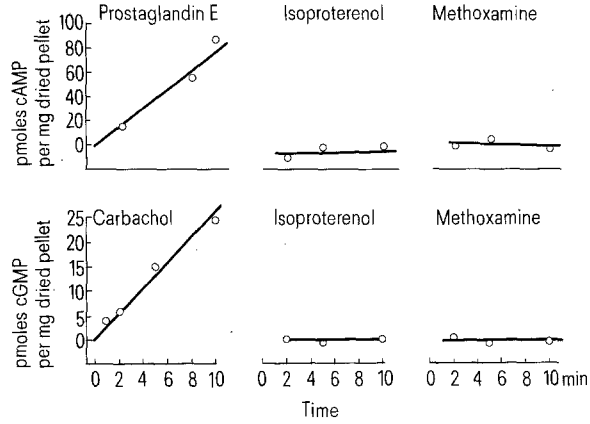
Methods. The seminal vesicle epithelium of mature guinea-pigs was isolated and pieces of it were incubated in a bicarbonate buffer as described[11]. In addition, the buffer contained 0.5 mM of 3-isobutyl-methyl-xanthine as phosphodiesterase inhibitor and 10 mM glucose as energy source. The experiments were started after a preincubation period of 30 min by adding to the experimental vessels 20 µl of H_2O containing the indicated drug. The experiments were terminated by adding 8 ml of ethanol to the incubation. The resulting mixture was homogenized in a tissue grinder. The homogenate was acidified with 50 µl of 2N formic acid and boiled for 10–15 min, in order to remove the CO_2 contained in the incubation buffer, before being transferred to a centrifuge tube. The tissue grinder was washed with 5 ml of 80% ethanol. The wash was further treated similarly to the homogenate. Wash and homogenate were combined and spun for 10 min at 9500× g. The resulting pellets were dried over night at 90 °C and weighed. CAMP and cGMP contained in the supernatant were separated from each other by the method of Gilman and Murad[12]. The fractions obtained were lyophylized and analyzed with commercially available competitive binding assays (cAMP kit Amersham/Searle; cGMP radioimmuno assay, Schwarz/Mann). The recovery was estimated by adding small amounts of (^{3}H)-cAMP and of (^{3}H)-cGMP to the homogenate and measuring the amount regained in the lyophylisate. The results were corrected for recovery and expressed as pmoles cyclic nucleotide (cNT) per mg dried pellet.

Results and discussion. First we incubated the tissue with either prostaglandin E_1 (PGE_1) or carbachol-HCl, 2 drugs known to activate specifically the cyclases which produce cAMP and cGMP, respectively[10,13]. The results demonstrated that the isolated epithelium contains both nucleotide cyclases in a form which can be activated by appropriated drugs in vitro (see figure). Next we investigated the effect of epinephrine-HCl, an adrenergic agoinst, which interacts with both alpha and beta receptors[14]. PGE_1 and carbachol-HCl were included as positive controls. Epinephrine-HCl had no effect on the accumulation of cNTs, indicating that the epithelium contains no functional adrenergic receptors (see table). This conclusion was confirmed in a last set of experiments in which isoproterenol-HCl and methoxamine-HCl, agonists for beta and alpha receptors, respectively, were employed. The experiment was executed as a time course study in order to detect even an early and transient accumulation of one or both of the cNTs. As can be seen in the figure, neither of the 2 drugs provoked any measurable response.

In vitro effect of different drugs on adenylate and guanylate cyclase activities present in the isolated seminal vesicle epithelium of the guinea-pig

	pmoles cAMP (mg dried pellet)	pmoles cGMP (mg dried pellet)
Control (n=4)	18.4±2.8*	2.7±0.8
PGE_1 (10^{-5} M, n=4)	**79.6±8.3	***3.2±0.6
Carbachol-HCl (10^{-5} M, n=4)	***21.9±5.7	**6.1±2.2
Epinephrine-HCl (10^{-5} M, n=4)	***22.3±3.8	***3.1±1.7

Seminal vesicle epithelium was taken from 4 animals. The tissue of each animal was divided into 4 equal parts which were incubated separately. This allowed for 1 control and 3 experimental incubations per animal, of which the later 3 were exposed to the indicated drugs for 10 min. The experiments were performed and assayed as described in the method section. The results were statistically analyzed, using the paired t-test method. *Mean ± SD. **Significantly different from control ($p < 0.02$). ***Not significantly different from control ($p \geq 0.05$).



Influence of different drugs on the time course of cyclic nucleotide accumulation. An experimental and a control incubation were performed for each time point. The amount of cyclic nucleotides contained in each incubation vessel was expressed as pmoles per mg of ethanol extracted and dried tissue. The difference between the amounts of cyclic nucleotides found in corresponding experimental and control incubation is shown. For details, see method section. Tissue from 4 and 2 animals was used for the incubations with PGE_1 and with the other drugs, respectively. The final concentration was 10^{-5} M, $7 \cdot 10^{-5}$ M, 10^{-4} M and 10^{-5} M for PGE_1, carbachol-HCl, isoproterenol-HCl and methoxamine-HCl, respectively.

This study implies that in vivo only cholinergic nerve fibres can influence the seminal vesicle epithelium of the guinea-pig. Therefore, if neuronal stimuli do modulate the secretory rate in this tissue, it could only be through the parasympathetic system and not through the sympathetic system.

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Toxic effect of cadmium nitrate on the liver of *Channa punctatus*

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Summary. Exposure of a fresh water fish, *Channa punctatus*, in a medium containing as low as 0.01 ppm of cadmium nitrate, resulted in the necrosis of hepatic cells. A temporary recovery of these cells was however observed when the animals were exposed to lower concentrations.

The advancement of civilization has resulted in an increasing technological use of heavy metals in industries. Cadmium for instance is widely used these days in the manufacture of alloys, pigments for paint and several other industries. This metal is known to pollute the aquatic environment and cause injury to the animals. The target organs often include the liver, the gastrointestinal tract, the respiratory tract and the kidney[2].

Recently Cherian et al.[3], Valberg et al.[4] and Hidalgo et al.[5] reported on the ill effects induced by cadmium on the renal tissue, the gastrointestinal tract and protein synthesis in liver respectively in rodents. Benoit et al.[6], working on the effect of cadmium on 3 generations of brook trout *(Salvelinus fontinalis)*, found that the growth of juvenile of second and third generation offspring was significantly retarded. However, information available on the toxic effects of cadmium on fishes is quite meagre. The present investigation was therefore undertaken to study toxicity induced by cadmium nitrate on the liver of an economically important freshwater fish, the snake-headed fish, *Ch. punctatus*.

Materials and methods. Only adult, healthy fishes of more or less the same size were selected as experimental animals. A number of glass aquaria each of 100:1 capacity were used for experimental work. One aquarium was maintained solely for normal fishes used as control. A stock solution of 1000 ppm of cadmium nitrate was prepared and from this, different quantities were added to other aquaria so as to bring the concentration of cadmium nitrate in the medium, respectively, to 0.01 ppm, 0.03 ppm and 0.05 ppm. About 30–35 laboratory acclimatized specimens of fishes were introduced into each aquarium. All aquaria were properly maintained and the fishes were fed regularly. After specific test periods, i.e. 1, 2, 4, 10, 17, 27, 39, 46, 51 days etc., a few fishes were sacrificed every time and pieces of liver from these animals were immediately fixed in neutral formalin for histopathological studies. The liver from a control fish was also fixed simultaneously. Following the standard technique, paraffin blocks were prepared. Sections of 2–3 µm thickness were taken and double stained with haematoxylin eosin. Some sections of the fresh frozen liver tissue were also stained with Sudan black B for the detection of the fat. The experiments were repeated thrice at random during different periods and a minimum of 3 animals were sacrificed every time.

Observations. Lipid in the form of droplets was observed only when the sections were specifically stained with Sudan black B in both the normal and treated animals. This was done to ascertain that the vacuolization in some sections was not due to the deposition of fat.

The histology of the liver of the normal fish. The liver is comprised of a continuous mass of large hexagonal cells forming laminae or cords. Suspended in the hepatic labyrinth of the laminae are seen a number of blood sinusoids. 2 cells thick wall separates adjacent laminae (figure 1).

The histology of the liver of the fish exposed to cadmium nitrate. On 24 h of exposure, even with the lowest concentration of cadmium nitrate, both the cytoplasmic and nuclear material started precipitating, resulting in the partial vacuolization of the cell. Gradually thereafter, a further reduction of the cytoplasmic material and shrinkage of the nuclei were noticed. After 13 days of exposure to 0.01 ppm and 0.03 ppm concentrations most of the hepatic cells became practically devoid of cytoplasm and vacuolization was more or less complete (figure 2). By this time the nuclei were observed to be at various stages of disintegration and closely pressed towards the border. Whatever was left of the nuclear material in the nuclei, it was seen precipitated towards the border.

Quite interestingly, the regeneration of the protoplasmic material was observed to have started by day 17 in fishes exposed to 0.01 ppm and by day 20 in fishes exposed to 0.03 ppm concentration. With the former concentration, both the cytoplasmic and nuclear material appeared normal by day 28 (figure 3). With 0.03 ppm concentration, the cell structure became normal by day 40. Very soon thereafter, the degeneration of the cellular contents started once again. By day 51, the protoplasmic material had degenerated more or less completely and the decomposed material was seen distributed at random throughout the cell remains.

With higher concentration, i.e. with 0.05 ppm of concentration, the degeneration of the tissue was severe and rapid. By day 20, most of the cells became devoid of protoplasmic contents (figure 4). By day 29, coagulate vacuolization was