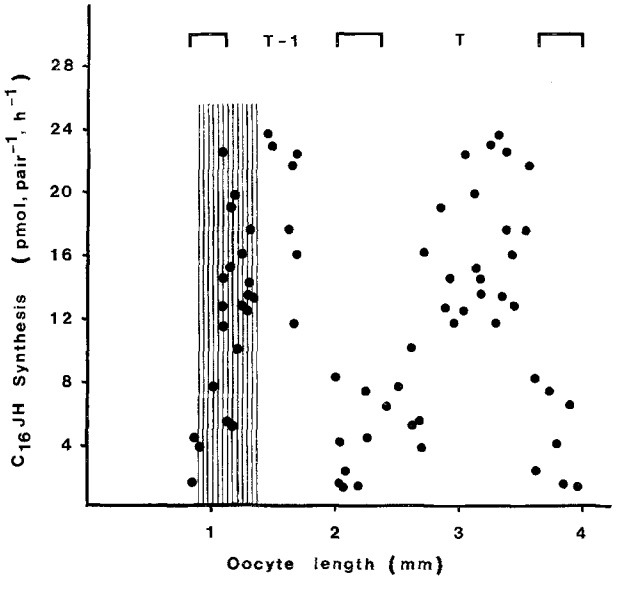
penultimate oocytes (previously T-2) are 0.8–1.0 mm long and are at the point of entry into vitellogenesis. Patency tests showed that T-1 oocytes entered vitellogenesis over the size range 0.9 to 1.3 mm. During the subsequent period the T-1 oocytes grow to a length of 2.0–2.3 mm and the T oocytes develop to a length of 3.7–4.0 mm, at which point the follicular epithelium deposits the chorion. The chorionated T oocytes are ovulated and the sequence is repeated. Vitellogenic oocytes are present at all times during the gonotrophic cycle, and, with the exception of a short period from the onset of chorionation until shortly after ovulation, two oocytes in each ovariole are forming yolk simultaneously. These results substantiate and elaborate previous reports by PRATT[8] and BELL[9], although the latter author found an oviposition cycle length of 5 days, possibly the effect of the use of a different diet.

Freshly excised corpora allata from adult female *P. americana* were found to exhibit a wide range of C_{16}JH biosynthetic ability. The validity of single time-point measurements of C_{16}JH production to estimate the rates of hormone biosynthesis in isolated corpora allata of *P. americana* has already been investigated[16]. These previous investigations also showed that the rate of release of C_{16}JH was strictly proportional to the rate of biosynthesis of the hormone, over a wide range of synthetic activities. The rates of C_{16}JH synthesis and release by individual pairs or split pairs range from 1–25 pmol/pair/h and when glandular activity was plotted against corresponding oocyte lengths a cyclic pattern was evident (Figure). Two cycles of corpus allatum activity occur during the vitellogenic growth of each wave of oocytes. At no time during the ongoing gonotrophic cycles have we found glands which were totally incapable of synthesizing and releasing C_{16}JH. In a significant number of animals (up to 6% of those sampled) the development of the T-1 oocyte was considerably retarded with respect to the T oocyte, and glands from these animals all had low synthetic rates; these data have been omitted from the Figure. These cycles of corpus allatum activity could be related to the development of either the T, T-1, or both T and T-1 oocytes. The close correlation that we find between the rise in corpus allatum activity and the onset of vitellogenesis of the T-1 oocyte, and the report that there are increases in the rates of protein accumulation by both T and T-1 oocytes in mid-cycle[9], suggest that juvenile hormone is necessary for both initiation and maintenance of vitellogenesis in this species[17].



Relationship between oocyte length and rate of synthesis of C_{16} juvenile hormone by single pairs or split pairs of corpora allata from adult female *Periplaneta americana*, as revealed by in vitro incorporation of [methyl-^{14}C]methionine. Brackets represent the stages in the growth of each wave of oocyte which can be related to the onset of ootheca formation in the gonotrophic cycle. Shaded area represents the size range over which oocytes (T-1) begin active vitellogenesis.

Zusammenfassung. Durch Inkorporation von ^{14}C-Methionin in die Corpora allata von *Periplaneta americana* werden während des Eireifungszyklus zwei Aktivitätsmaxima festgestellt und diese als Juvenilhormonsynthese interpretiert.

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In vivo Effects of Acetylcholine on LH Secretion[1]

It has recently been shown that acetylcholine (Ach) is able to liberate the FSH-Releasing Hormone (FSH-RH) and the LH-Releasing Hormone (LH-RH) from rat hypothalamic fragments incubated in vitro in the presence of anterior pituitary tissue[2,3]. This effect of Ach can be respectively enhanced or depressed by the presence in the incubation medium of prostigmine (an anti-acetylcholinesterase drug, which potentiates the activity of Ach) or of atropine (a blocker of cholinergic receptors). These data have provided additional[4–6] evidence for a role of cholinergic mechanisms in the processes controlling the secretion of pituitary gonadotropins. The present experiments have been planned in order to verify whether cholinergic inputs might participate in the regulation of LH secretion in vivo.

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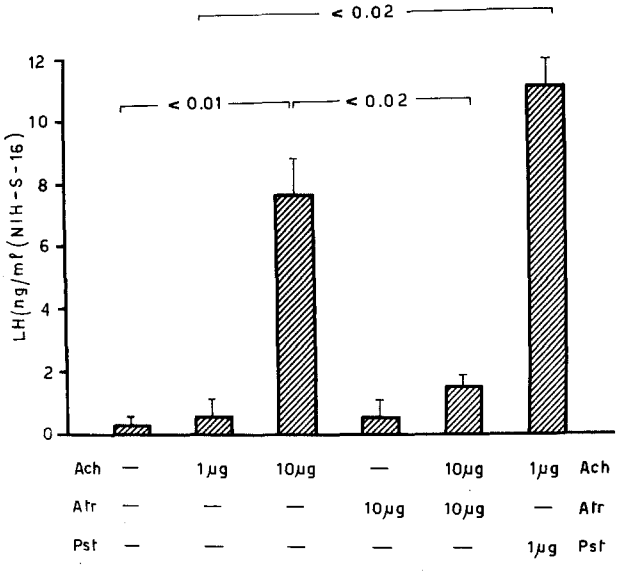
Materials and methods. Adult male Sprague-Dawley rats, weighing 150–180 g, were used. Animals were caged in rooms with controlled temperature and humidity (lights on from 06.30 to 20.30), and were fed with a standard pellet diet; water was given ad libitum. All animals were implanted, into a lateral ventricle of the brain, with a cannula which was cemented to the skull to allow the administration of substances into the cerebrospinal fluid (CSF)[7]. 1 week after implantation of the cannula, the rats were anesthetized with Nembutal (40 mg/kg). Ach, atropine and prostigmine were administered into the cannula in the doses indicated in the Figure and in the volume of 20 µl. A Hamilton microsyringe was used. Blood was collected from the jugular vein 15 min after injection. Serum LH was evaluated by the radioimmunoassay of NISWENDER et al.[8]. The statistical analysis of the data was performed using Student's *t*-test.

Results. Each bar in the Figure represents the mean of measurements performed in at least 5 animals. It is clear that Ach injected into the cerebral ventricles is able to induce a significant release of LH. However, the effect of Ach appears only when the dose of 10 µg is used. A smaller amount (1 µg) is totally inactive. Atropine, when given together with Ach, proves able to block the LH-releasing activity of the cholinergic mediator. There was no significant rise in serum levels of LH in the animals receiving intraventricular injections of 10 µg of Ach coupled with 10 µg of atropine. On the contrary, prostigmine potentiates the LH-releasing activity of Ach. The dose of 1 µg of Ach, which was ineffective when given alone, became even more active than the dose of 10 µg of Ach, when given intraventricularly in conjunction with 1 µg of prostigmine.

Discussion. The in vivo data here presented suggest that Ach may play a significant role in the control of LH secretion. This hypothesis is supported by some recent indirect observations. It has been reported that intrahypothalamic implants of atropine inhibit, in the female rat, the ovarian compensatory hypertrophy which normally follows unilateral ovariectomy, and induce a decrease of ovarian weight in intact animals[9,10]. Moreover, atropine (administered either s.c. or into the 3rd ventricle) blocks the proestrous surge of LH and of FSH secretion and prevents the elevation of plasma gonadotropins which normally occurs following castration[11]. Finally, systemic injections of the cholinominetic drug pilocarpine increase LH release[12].

In order to explain the in vitro effects of Ach on the release of LH-RH and FSH-RH, it has been proposed that Ach might: 1. operate as the neurotransmitter of extrahypothalamic fibres impinging on the hypothalamic cells which synthetize the gonadotropin-releasing hormones; or 2. facilitate the liberation of the gonadotropin-releasing hormones, via a direct action on the nerve terminals at the level of the median eminence[2,3]. The in vivo experiments here reported do not provide a clear cut answer on the mode of action of Ach. However, it is important to note that recent findings underline a major role for ependymal cells and for tanycytes[13,14] in the transport of substances from the CSF to the median eminence. If Ach is picked up and transported to the median eminence by these elements, the data here presented might be taken as a proof of the fact that the neurotransmitter acts on the nerve terminals where the releasing hormones are stored (hypothesis No. 2). It is indeed inconceivable that Ach might be transported to reach the arcuate-ventromedial region, where the cell bodies which synthesize LH-RH are mainly located[15]. It is also possible, however, that Ach might stimulate some of the circumventricular structures (organum vasculosum laminae terminalis?) which accumulate LH-RH and which may play a role in the control of gonadotropin secretion[16].

The data here presented do not allow any definite conclusion on whether the stimulatory effect exerted by Ach on the release of LH is muscarinic or nicotinic in nature. There are, however, some reasons for arguing that the effects here reported are mainly of the muscarine type. First of all, the stimulatory effect of Ach could be antagonized by atropine, which is a typical blocker of muscarinic receptors. Moreover, nicotine and antinicotinic drugs have been reported respectively to inhibit and stimulate gonadotropin secretion[17–20].



Effect of intraventricular injection of acetylcholine (Ach) of atropine (Atr) and of prostigmine (Pst) on serum LH in normal male rats.

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Résumé. L'injection intraventriculaire d'acetylcholine provoque chez le rat mâle une sécrétion accrue de LH. Cet effet de l'acetylcholine est inhibé par l'administration intraventriculaire d'atropine, et est augmenté par l'injection intraventriculaire de prostigmine.

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Purification of Rabbit Angiotensin II-Antibodies by Affinity Chromatography

Antibodies against angiotensin II have been used repeatedly for in vivo experiments, in which the participation of the renin-angiotensin system in various forms of experimental hypertension was studied (for review see CHRISTLIEB and HICKLER[1]). In most of these studies crude antisera have been injected into the test animal with the obvious disadvantage that not only the antibody, but also considerable amounts of heterologous protein, renin, renin substrate and angiotensin II (up to 300 ng/ml) were injected. In order to provide a more defined basis for in vivo studies, the conditions for purification of angiotensin-antibodies by affinity chromatography were studied.

Materials and methods. Anti-sera were raised in male white New Zealand rabbits, weighing 2000–2500 g, by immunization with asp[1]-ileu[5]-angiotensin II (Schwarz/Mann, Orangeburg, USA) coupled to porcine γ-globulin by the carbodiimide method, as described by GOODFRIEND et al.[3]. After 3 to 5 months all animals developed antibody titers ranging between 1:13000 and 1:180000.

Affinity columns for most of the separation experiments were prepared by coupling angiotensin II to the sepharose matrix via albumin as a spacer, a principle first proposed by CUATRECASAS et al.[4]. In a typical experiment 3 g of cyanogenbromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) were washed in 1 m*M tris*-HCl buffer pH 6.0, and adjusted to pH 8.0 with $NaHCO_3$ buffer. Bovine serum albumin (Behring Werke, Marburg), 20 mg in 0.1 *M* $NaHCO_3$ buffer pH 8.0, containing 0.5 *M* NaCl, was added. After 2 h of incubation, about 65% of the albumin had been bound to the sepharose as judged by the decrease in absorbancy at 280 nm and protein content in the supernatant[5]. Following treatment with 1 *M* ethanolamine at pH 8 for 2 h, the product was washed 4 times alternately with 0.1 *M* borate buffer pH 8.0, and 0.1 *M* acetate buffer pH 4.0, and finally adjusted to pH 6.0. subsequently 2 mg angiotensin II were coupled to the sepharose bound albumin by the addition of 1.2 g of N-ethyl-N-dimethyl-aminopropyl-carbodiimide at pH 6.0. A small amount of I^{125}-labelled angiotensin II was added to the reaction mixture, and the radioactivity of the sepharose-free supernatant was determined at various time intervals. Furthermore, the amount of angiotensin II remaining in the supernatant was estimated by rat blood pressure bioassay[6]. The coupling product was again washed alternately with acidic and alkaline buffer was described above, and packed in a 1.1×21 cm column and equilibrated with 0.1 *M* tris-HCl pH 7.4. Angiotensin II-antiserum (9 ml) was diluted 1:3 with the same buffer and poured through the column at a flow rate of about 0.5 ml/min. The column was washed with 50 ml 0.1 *M* tris-HCl pH 7.4 and eluted in batches with 50 ml each of 0.1 *M* Na-citrate-HCl buffer of pH 5, 4, 3, 2, 1.2 and finally pH 1.2 containing 0.5 *M* NaCl. The eluate was collected in 10 ml fractions into tubes kept at 0° and containing 2 ml 3 *M tris*-HCl pH 7.4, to immediately neutralize the acidic eluate.

In a separate experiment angiotensin II was coupled directly to CNBr-activated Sepharose without using albumin as a spacer.

Antibody concentrations were estimated by titer determination. The titer is arbitrarily defined as the dilution of an antibody preparation, at which exactly half of 10 pg of I^{125}-angiotensin II is bound to the antibody in a standard radioimmunoassay system[7] (250 µl of 0.1 *M tris*-acetate buffer pH 7.4 with 0.1% human serum albumin, 20 h equilibration at 0°). Assuming that under these conditions of extreme dilution (e.g. antiserum is diluted at least 15000-fold), there is no interference by substances present in the original material, the amount of antibody which binds 5 pg of angiotensin II can be taken as an arbitrary antibody unit, provided that no significant differences in affinity exist. Thus, the antibody concentration of a given preparation can be expressed as the reciprocal of the titer. The radioimmunoassay system for angiotensin II and the estimation of cross reactivity with analogous peptides has been described previously[7]. The apparent affinity constants of the antibody preparations were estimated from double-reciprocal plots according to NISONOFF and PRESSMAN[8].

Results and discussion. The coupling of angiotensin II to the sepharose-albumin complex appeared to be complete after 2 h of incubation, since at that time there was no detectable biological activity in the supernatant, as judged by rat blood pressure assay. However, at the same time, half of the radioactivity of I^{125}-labelled angiotensin II, which had been added at the beginning of the coupling reaction, was still in the soluble form. This discrepancy between biological activity and radioactivity may find its explanation in a partial polymerization of angiotensin, i.e. the formation of dimers or trimers of angiotensin II by the carbodiimid reaction, since the immobilized albumin is less readily available as a reaction partner. This interpretation is supported by the following experiment. The product of a reaction mixture identical

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