Testicular GH receptors during the spermatogenic cycle in fish

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The octadecaneuropeptide (ODN) derives from the endogenous ligand of benzodiazepine receptors (BZR) called diazepam-binding inhibitor (DBI). Cultured rat astrocytes contain and release substantial amounts of DBI-related peptides, and ODN acts as an autocrine factor controlling the intracellular calcium concentration ($[Ca^{2+}]_i$) in astrocytes. The purpose of this study was to determine the type of receptor involved in the response of astrocytes to ODN. The stimulatory effect of ODN (10^{-8} M) on $[Ca^{2+}]_i$ was not affected by the central-type BZR antagonist flumazenil (10^{-6} M) or by the GABAA receptor antagonist SR 95531 (10^{-6} M). Peripheral-type BZR antagonist PK 11195 (10^{-6} M) did not block the ODN-induced $[Ca^{2+}]_i$ increase. PK 11195 (10^{-8} M), as well as the peripheral-type BZR ligands Ro5-4864 and flunitrazepam (10^{-8} M each), mimicked the effect of ODN on $[Ca^{2+}]_i$. However, ODN did not affect the [^{3}H] flunitrazepam binding, suggesting that ODN acts on astrocytes through a novel type of receptor. Suppression of calcium in the incubation medium did not block the action of ODN on $[Ca^{2+}]_i$. In contrast, thapsigargin (10^{-6} M) totally suppressed the ODN-induced $[Ca^{2+}]_i$ increase. ODN (10^{-8} M) induced an increase in IP3 formation and a concomittant decrease in PIP2 concentration.

Pretreatment of astrocytes with PTX (0.2 µg/ml, 4 h) totally suppressed the stimulatory effect of ODN on both phosphatidylinositol breakdown and Ca^{2+} mobilization. Taken together, these data suggest that, in rat astrocytes, the stimulatory effect of ODN on $[Ca^{2+}]_i$ is mediated through a high affinity receptor coupled to a phospholipase C via a pertussis toxin-sensitive G protein. *Supported by INSERM, the European Union (C.H.M. # ERB-CHRXCT920017), ORIL and the Conseil Régional de Haute-Normandie.*

P176 TROUT BRADYKININ STIMULATES THE GASTRIC MOTILITY OF TROUT VIA A NOVEL TYPE OF BRADYKININ RECEPTOR

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In mammals, the effects of bradykinin (BK) are mediated through B_2 receptors, at which BK and Lys^{0}BK are equipotent but are > 100-fold more potent than des-Arg^{9}BK, or through B_1 receptors at which des-Arg^{9}BK is more potent than BK. Trout BK ([Arg^0, Trp^5, Leu^8]BK), recently isolated from the plasma of the rainbow trout, produced concentration-dependent contractions of isolated longitudinal muscle strips from the trout stomach ($pD_2 = 7.42 \pm 0.03$). Effects were attenuated by indomethacin, indicating that the response in part is mediated by prostaglandins. The response was not affected by the B_1 antagonist, des-Arg^9, [Leu^8]BK. The potent B_2 antagonist Hoe 140 competitively inhibited the action of trout BK but also showed agonistic actions (57 ± 15 % of the maximum response of trout BK ; pD_2 7.44 ± 0.12). Mammalian BK had no effect on the motility and des-Arg^0 trout BK had only a very weak effect and was > 100-fold less potent than trout BK. Des-Arg^{10} trout BK was on the other hand a relatively good agonist ($pD_2 = 6.80 \pm 0.03$ with 55 ± 7 % of the maximum response of trout BK). The data indicate that the bradykinin receptor in trout stomach has pharmacological properties that are different from the receptors identified in mammals.

P177 TESTICULAR GH RECEPTORS DURING THE SPERMATOGENIC CYCLE IN FISH

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Evidence for growth hormone (GH) binding to specific receptors in trout testes and for GH involvement in the regulation of fish gonadal functions have been obtained in vivo and *in vitro* (review : Le Gac et al. 1993). This work was undertaken to set up a method for characterization and quantitative evaluation of GH receptors at all stages of the testicular development in trout *(Oncorhynchus mykiss)* and to describe the evolution of GH binding affinity (Ka) and capacity (Bmax) during one spermatogenetic cycle (12 sampling times between February and November). Ka and Bmax were determined by saturation analysis using labeled recombinant trout GH and crude particulate preparations obtained from pools of gonads at the same histological stage of spermatogenesis. Ka varies slightly during the cycle between 3.0 and 6.2×10^9 M^{-1}. The receptor concentration, expressed in fmoles per mg protein or per gram of tissue is high during the immature stage, when the testis consists mainly of somatic cells + limited number of A spermatogonia (580 fmoles/g tissue). It decreases regularly from the beginning of spermatogenesis (B gonia proliferation : 730 fmoles/g tissue) to spawning (testis essentially composed of spermiogenic germ cells and spermatozoa : 242 fmoles/g tissue). Conversely the absolute testicular capacity increases dramatically between stages III and VII (499 to 22 880 fmoles/2 gonads), a developmental period where testicular growth is extremely rapid. The data analysis is still going on and the possible physiological meaning of these results will be discussed at the congress.

P178 PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE : A KEY PLAYER IN MALE REPRODUCTION ?

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The rat testis contains high amount of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)-like immunoreactivity (PACAP-LI). The specific PACAP-binding sites are localized in the seminiferous tubules by autoradiography. The present study was aimed to examine the precise distribution, ultrastructural localization of PACAP and its receptor (PACAPR) and these transcripts in the rat testis by immunocytochemistry and *in situ* hybridization. PACAP-LI was detected immunocytochemically in the developing germ cells but not in either Sertoli or Leydig cells. Intense PACAP-LI was detected in spermatids situated near the lumen of the tubules. In spermatids, PACAP-LI was detected during the cap phase and acrosome phase, but not in the maturation phase. At the ultrastructural level, PACAP-LI was found in acrosomal granules and acrosomal caps of spermatids. *In situ* hybridization indicated that most of the signal was detected especially in spermatogonia and primary spermatocytes, indicating that PACAP gene transcription occurs in early developing germ cells. PACAPR showed specific immunostaining in spermatids during early developmental stages when expression of PACAP reaches its peak. High-level of PACAPR mRNA expression was found in spermatogonia, primary spermatocytes and spermatids. These findings further support the view that PACAP and PACAPR are synthesized in germ cells and participates in spermatogenesis, particularly spermiogenesis, probably by autocrine and paracrine mechanism. (Supported in part by NIH and Ministry of Education, Science, and Culture of Japan)

P179 THE STIMULATORY EFFECT OF ENDOTHELIN-1 ON FROG CORTICOSTEROID SECRETION IS MEDIATED THROUGH AN ET_A RECEPTOR SUBTYPE

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We have previously shown that endothelin-1 (ET-1) stimulates *in vitro* the secretion of corticosterone and aldosterone from the frog adrenal gland. The aim of the present study was to investigate the mechanism of action of ET-1 on adrenocortical cells. Various antagonists were used to determine the type of receptor involved in the action of ET-1 on perifused frog interrenal slices. Bosentan, a non selective ET_A/ET_B receptor antagonist, totally blocked the ET-1 evoked stimulation of corticosterone and aldosterone secretion. The selective ET_A receptor antagonist BQ-485 also inhibited the response of frog adrenocotical cells to ET-1. In contrast, the selective ET_B receptor antagonist IRL 1038 did not affect the response of the adrenal gland to ET-1. In addition, the ET_B receptor agonist IRL 1620 did not mimic the stimulatory effect of ET-1 on corticosteroid secretion. Incubation of frog adrenal tissue with ET-1 induced a dose-dependent increase in the formation of total inositol phosphates. Microfluorimetric measurements indicated that ET-1 induced a rapid and transient rise of cytosolic calcium concentrations in adrenocortical cells. The present study demonstrates that the corticotropic action of ET-1 on the frog adrenal gland is mediated by an ET_A receptor subtype positively coupled to phospholipase C. *Supported by DRET (92-099) and the Conseil Regional de Haute-Normandie.*