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Possible sites of dopaminergic inhibition of gonadotropin release from the pituitary of a teleost fish, tilapia☆

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Abstract

The present study is an attempt to find sites of dopaminergic inhibition along the transduction cascades culminating in gonadotropin (GtH) release in a teleost fish, tilapia. Experiments were carried out on perifused pituitary fragments and in primary culture of trypsinized pituitary cells. Salmon GnRH, chicken GnRH I and II stimulated GtH release in culture with estimated ED_{50} values of 15.56 pM, 2.55 nM and 8.65 pM, respectively. Apomorphine (APO; 1 μM) totally abolished this stimulation. Dopamine (DA; 1 μM) reduced both basal and GnRHa-stimulated GtH release from perifused pituitary fragments but did not alter the formation of cAMP. In a similar perifusion experiment DA abolished GtH release in response to forskolin (10 μM) with no reduction in cAMP formation. This indicates that one site of the dopaminergic inhibition is distal to cAMP formation, an indication not compatible with the classic characteristic of DA D_2 type mode of action. The inhibition of GtH release in culture, caused by 1 μM APO, the specific DA D_2 agonists LY 171555 (LY) or bromocryptine (BRCR) could not be reversed by activating protein kinase C (PKC) by DiC8 or the phorbol ester TPA. This would indicate a site for DA action distal to PKC. However, the stimulatory effect of arachidonic acid (AA; 50 μM) in perifusion was not reduced by DA (1 μM) or by APO, LY or BRCR in culture, which suggests a site for DA action proximal to AA formation. APO, LY and BRCR reduced GtH release in response to the Ca^{2+} ionophore A23187, however, their inhibitory effect was reversed by 10 μM ionomycin. The stimulatory effect of ionophore A23187 differed from that of ionomycin in that A23187 was able to stimulate GtH release only in the presence of Ca^{2+} in the medium, whereas ionomycin stimulated the release also in the absence of the ion, and even in the presence of EGTA. It is assumed that ionomycin at 10 μM promotes the mobilization of Ca^{2+} from intracellular stores. As neither ionomycin nor AA caused any leakage of lactic dehydrogenase from cultured pituitary cells, the GtH released in response to this agonist is specific and cannot be attributed to damage of the cells' membrane. It is proposed that, in addition to sites distal to cAMP formation and proximal to AA formation, DA may inhibit GtH release from the pituitary of tilapia at sites distal to Ca^{2+} influx and at a step proximal to Ca^{2+} mobilization from intracellular sources.

Keywords: Tilapia; Pituitary; Gonadotropin; Salmon gonadotropin; Dopamine; Ca^{2+} influx; Mobilization; Apomorphine; A23187; Ionomycin; 1,2-Dioctanoyl-*sn*-glycerol (DiC8); cAMP; Forskolin; Quinpirole; Bromocryptine; Arachidonic acid

1. Introduction

Gonadotropin (GtH) secretion from the teleost pituitary is regulated by a multitude of hypothalamic agents including GABA, norepinephrine, NPY and serotonin which reach the gonadotrophs directly through nerve tracts from the hypothalamus (reviewed by Kah et al., 1993). However, the main regulators of GtH release are gonadotropin-releasing hormone (GnRH) in its various forms, which stimulates GtH release, and dopamine (DA) which inhibits the release (reviewed by Peter et al., 1986; 1991). The most common forms of GnRH in fish are the chicken GnRH-II ([His^5,Trp^7,Tyr^8]-GnRH; cGnRH-II) and salmon GnRH ([Trp^7,Leu^8]-GnRH; sGnRH) (Sherwood and Coe, 1991).

DA inhibition of GtH secretion is dominant in the carp (Lin et al., 1988), the goldfish (Chang et al., 1984a,b) and the African catfish (de Leeuw et al., 1985, 1986). However, the inhibitory effect of DA on GtH release is less obvious in other fish such as the coho salmon (Van Der Kraak et al., 1986) and the gilthead seabream (Zohar et al., 1987), and is entirely absent in the Atlantic croaker

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(Copeland and Thomas, 1989). In vivo experiments in tilapia showed that several DA receptor antagonists such as pimozide, metoclopramide or domperidone augment the stimulatory effect of a superactive GnRH analog and induced spawning in females (Gissis et al., 1991).

Previous studies in this laboratory have shown that the stimulatory effect of GnRH on GtH release from the pituitary of tilapia is mediated, as in mammals, through a cascade of intracellular events spanning the influx of Ca^{2+}, activation of protein kinase C (PKC), and arachidonic acid (AA) or its metabolites (Levavi-Sivan and Yaron, 1989, 1993). In addition, evidence has been presented that in tilapia, adenylate cyclase and cAMP also take part in the mediation as a parallel or an interconnected transduction system (Levavi-Sivan and Yaron, 1991, 1992).

In the goldfish, the dopamine D_2 receptor agonist, bromocryptine, depresses the GnRHa-stimulated GtH elevation in the serum, while the DA D_2 receptor antagonists such as pimozide or metoclopramide increase plasma GtH levels (Chang et al., 1984b). The specific binding of [^{3}H]spiperone to pituitary membrane preparation of the goldfish and the African catfish was displaced by DA and specific D_2 agonists and antagonists (Omeljaniuk and Peter, 1989; Van Asselt et al., 1990). This has led to the conclusion that the dopaminergic inhibition on GtH release in fish is mediated through a DA D_2 type receptor.

It was suggested that DA down-regulates GnRH receptors in the catfish (De Leeuw et al., 1988). However, the onset of this effect required 2 h, a time-lag not compatible with the immediate effect of DA observed in vivo or in vitro (de Leeuw et al., 1985; Omeljaniuk et al., 1989, Yaron and Levavi-Sivan, 1991). Information on DA effects in fish pituitary beyond the receptor level is incomplete. The present research is an attempt to reveal sites of dopaminergic inhibition along the GnRH transduction cascades.

2. Materials and methods

2.1. Fish

Tilapia hybrids (*Oreochromis niloticus* × *O. aureus*), 80–100 g body weight, were collected at various times of the year from the fish farm of Kibbutz HaMaapil. The fish were kept in tanks at 18°C with a photoperiod of 8 h light/16 h dark; conditions which do not promote gonadal recrudescence. For maximal response in static culture studies fish were transferred to 26°C for 1 week before each experiment (Levavi-Sivan and Yaron, 1993).

2.2. Culture of dispersed pituitary cells

Pituitary cells were cultured according to Levavi-Sivan and Yaron (1992). Briefly, pituitary cells were dispersed by trypsinization and were plated on 24 multiwell plates (Corning, Coming NY) at a density of 2.5×10^5 cells/ml per well. The cells were cultured for 4 days under an atmosphere of 95% O_2 and 5% CO_2 and saturated humidity at 28°C. The cells were incubated with the DA agonists 15 min before adding the secretagogues. Following a further incubation of 3 h, 0.7 ml of the medium was removed and stored at –20°C until assayed.

When Ca^{2+}-free medium was used, the cells were plated and cultured for 4 days as above, and before manipulation they were rinsed with buffered physiological saline (BPS; 130 mM NaCl, 1.2 mM K_2HPO_4, 2.5 mM $MgSO_4$, 11 mM glucose, 1.2 mM $CaCl_2$, 50 mg% bovine serum albumin and 10 mM Pipes, pH 7.4). The control wells were exposed to ionophores A23187 or ionomycin in BPS only; parallel wells were exposed to the ionophores in Ca^{2+}-free BPS to which 1.2 mM NaCl was added to keep the same osmolality (300 mOsm). Another group of wells was exposed to the ionophores in Ca^{2+}-free BPS with ethylene bis-(oxythelenitrilo)-tetraacetic acid (EGTA; 0.2 mM).

The results, expressed as ng GtH release/well (mean ± SEM; $n = 4$) were analyzed by one-way ANOVA followed by a simultaneous comparison among means using least significant difference (LSD) test. Experiments of the same type were repeated 3–5 times and a representative experiment is shown. The analysis of curves and the ED_{50} values were calculated using a curve fitting program (ALLFIT; DeLean et al., 1978).

Plasma membrane integrity of cultured cells stimulated by ionomycin (10 μM) or arachidonic acid (100 μM) was assessed by determination of lactic dehydrogenase (LDH) activity using spectrophotometric monitoring of NADH breakdown.

2.3. Perifusion system

The perifusion method was according to Levavi-Sivan and Yaron, (1989). Briefly, pituitaries were excised from three fish and cut into fragments. The fragments were embedded in Biogel P-2 and perifused for 20 h at 27°C ± 1°C with Eagle's basal medium before the beginning of the experiments. The average GtH secretion rate during the last 3 h was considered as baseline. Two hours following the introduction of DA, the agonists were introduced in a 5-min pulse, and after an additional 2 h, the DA was withdrawn. The results are expressed as mean ± SEM of the ratio between the secretion rate after manipulation and the basal secretion rate.

Experiments were repeated 3–5 times and a representative experiment is shown.

2.4. Radioimmunoassays

The gonadotropin of tilapia (taGtH) was determined by a homologous radioimmunoassay as previously described (Levavi-Sivan and Yaron, 1992). Since the standard GtH used in this study was isolated and purified from pituitaries of adult fish collected during the spawning season

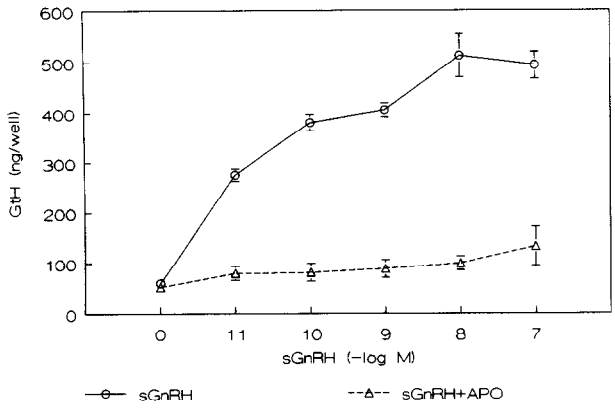

Fig. 1. GtH release from primary culture of dispersed pituitary cells of tilapia in response to graded doses of sGnRH in the presence or absence of 1 μM APO. Cells were dispersed by trypsinization and were plated (2.5×10^5 cells/well) in 1 ml of medium. The cells were cultured for 4 days at 28°C and were then challenged for 3 h with the agonist. APO was added 15 min prior to the GnRH. The release of GtH is expressed as ng/well (mean ± SEM; $n = 4$).

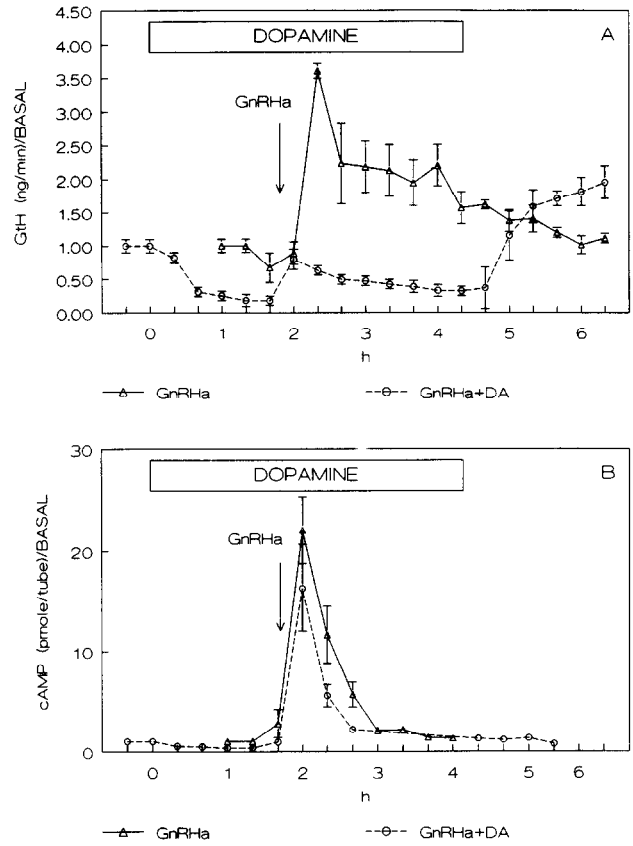

Fig. 2. GtH release (A) and cAMP concentration (B) in the effluent medium of perifused tilapia pituitary fragments in response to GnRHa ([D-Ala^6, Pro^9-NEt]-LHRH) in the presence or absence of 1 μM dopamine (DA). Pituitary fragments were perifused for 19 h at 27 ± 1°C and then exposed to DA for 2 h; they were then stimulated by GnRHa (100 nM) for 5 min and were perifused for an additional 2 h in the presence of DA. Data are presented as the mean ± SEM ($n = 3$) of the ratio between the secretion rate after stimulation and the basal secretion rate.

(Bogomolnaya et al., 1989), it is assumed that taGtH (henceforth GtH) corresponds to GtH II as defined in the salmon (reviewed by Kawauchi et al., 1989).

cAMP was determined as previously described by Levavi-Sivan and Yaron (1992). Briefly, the effluent medium was loaded on AMPREP minicolumns and the nucleotide was eluted. The eluate was lyophilized and the dry sample was redissolved in the assay buffer (0.05 M acetate buffer, pH 5.8). cAMP concentration was determined by the Amersham ^{125}I-labelled cAMP assay system following acetylation.

2.5. Reagents and drugs

Stock solutions (in 0.1 M acetic acid) of [Trp^7,Leu^8]-GnRH (salmon GnRH; sGnRH; Sigma), [His^5,Trp^7,Tyr^8]-GnRH (chicken GnRH-II; cGnRH-II) and [Gln^8]-GnRH (chicken GnRH-I; cGnRH-I; Millar, R.P. Cape Town), and [D-Ala^6,Pro^9-NEt]-GnRH (GnRH analog; GnRHa; Sigma, St. Louis, MO) were stored at −20°C and diluted in the test medium immediately prior to use. Apomorphine hydrochloride (RBI, Natick, USA) and dopamine (Sigma, St. Louis, MO) solutions were freshly prepared in the medium just before use. The D_2 agonist LY 171555 (quinpirole; 4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline hydrochloride; RBI, Natic, USA); ionomycin, 1,2-dioctanoyl-*sn*-glycerol (Di-C8, C8:0)) and 1-*O*-tetradecanoyl phorbol-13-acetate (TPA; Sigma, St. Louis, MO) were dissolved in dimethyl sulfoxide. The D_2 agonist bromocryptine (2α-Br-ergocryptine), the Ca^{2+} ionophore A23187, and arachidonic acid (Sigma St. Louis, MO) were dissolved in ethanol. Final concentrations of the solvents were less than 0.1% which did not alter basal GtH release.

3. Results

Exposure of dispersed tilapia pituitary cells to sGnRH, cGnRH-I and cGnRH-II for 3 h resulted in increased GtH release in a dose-dependent manner. The maximal response to all these peptides was similar (493.97 ± 26.32, 473.24 ± 5.26 and 525.63 ± 22.59 for sGnRH, cGnRH-I and cGnRH-II, respectively). However, they differed in their estimated ED_{50} values (15.6 ± 12.8 pM, 2.55 ± 2.5 nM and 8.65 ± 7.1 pM (mean ± SEM) for sGnRH, cGnRH-I and cGnRH-II, respectively). In the presence of 1 μM apomorphine (APO) the basal release was not significantly altered, however the drug totally inhibited the response to all GnRH forms (Fig. 1 presents the response to sGnRH and its inhibition by APO).

GtH secretion from perifused pituitary fragments in response to a pulse of GnRHa was totally abolished by 1 μM DA, and a rebound release above the basal level occurred after its withdrawal (Fig. 2a). However, the formation of cAMP by the same pituitary fragments, as reflected by its concentration in the effluent medium, was not impaired in the presence of the amine (Fig. 2b).

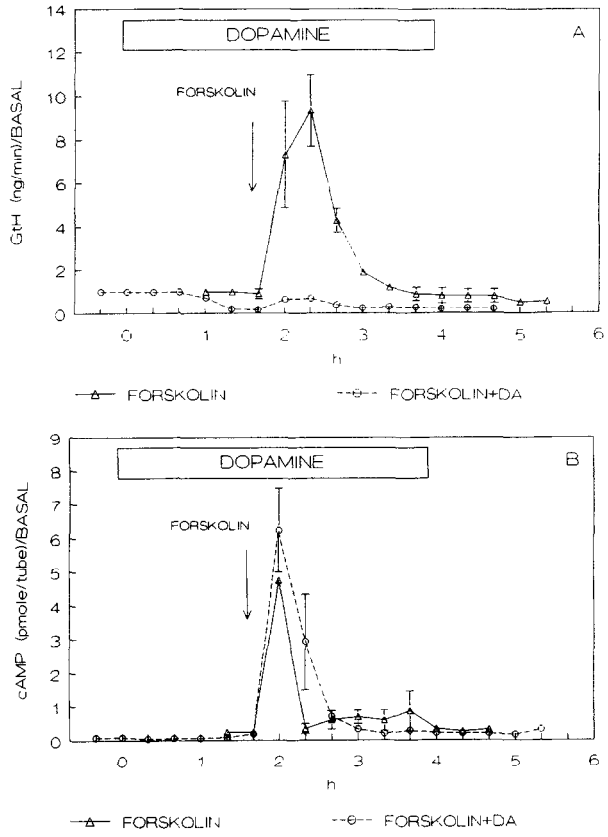

Fig. 3. GtH release (A) and cAMP concentration (B) in the effluent medium of perifused tilapia pituitary fragments in response to 10 μM forskolin in the presence or absence of 1 μM dopamine (DA). Other details are as in Fig. 2.

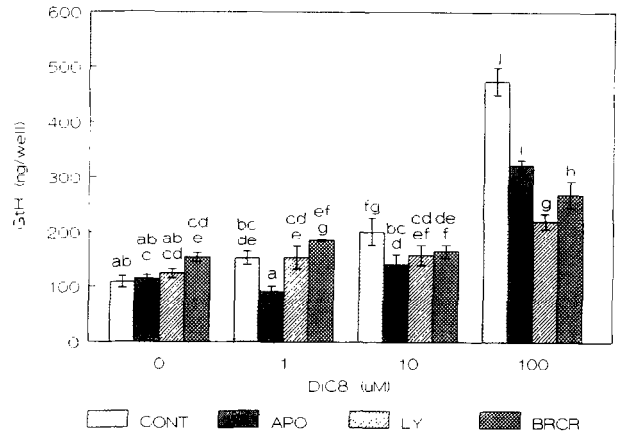

Fig. 4. GtH release from primary culture of dispersed pituitary cells in response to graded doses of DiC8 in the absence (CONT), or the presence of 1 μM APO, LY or BRCR. Other details are as in Fig. 1. The difference among means was analyzed by one-way ANOVA followed

Similarly, the increase in cAMP in response to 10 μM forskolin was not altered by the presence of 1 μM DA but the increase in GtH was totally abolished (Fig. 3).

The diacylglycerol analog DiC8 stimulated the release of GtH from cultured pituitary cells in a dose-dependent fashion (Fig. 4). APO and the DA D_2 receptor agonists LY 17155 (LY) and bromocryptine (BRCR), all at a concentration of 1 μM, inhibited the stimulation of GtH release in response to DiC8 at 10 μM or 100 μM (Fig. 4). The phorbol ester TPA stimulated GtH release from cultured pituitary cells in a dose-dependent manner in the

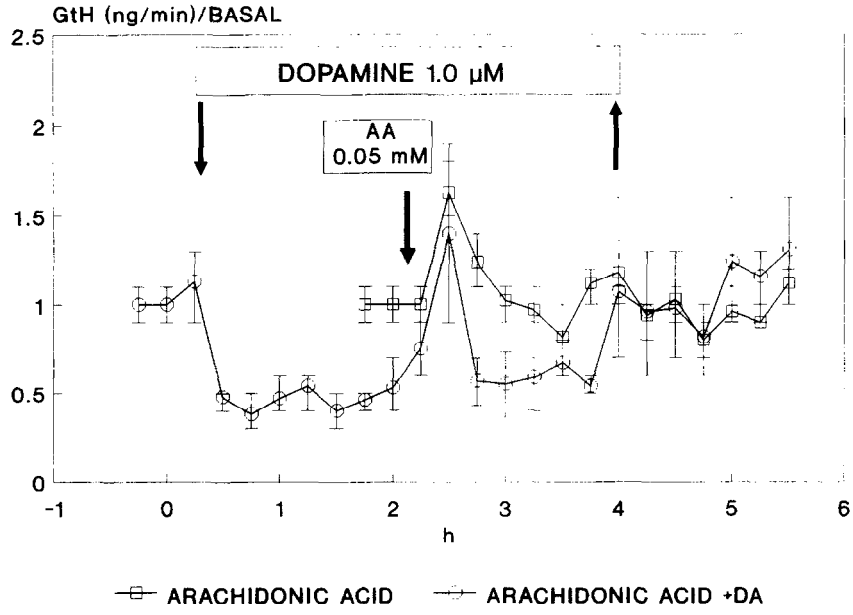

Fig. 5. GtH release in the effluent medium of perifused pituitary fragments in response to AA in the presence or absence of 1 μM DA. Other details are as in Fig. 2.

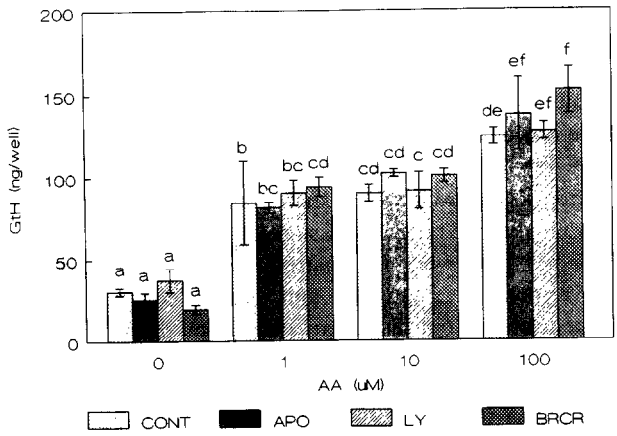

Fig. 6. GtH release from primary culture of dispersed pituitary cells in response to graded doses of AA in the absence (CONT) or presence of 1 μM APO, LY or BRCR. Culture details are as in Fig. 1. The presentation of data and their statistical analysis are as in Fig. 4.

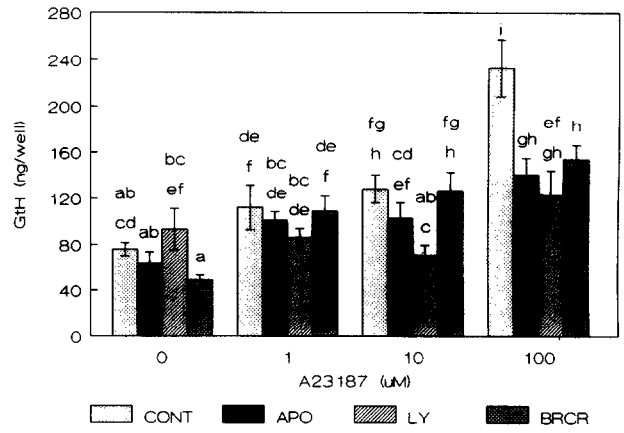

Fig. 8. GtH release from primary culture of dispersed pituitary cells in response to graded doses of A23187 in the absence (CONT) or presence of 1 μM of APO, LY or BRCR. Other details are as in Fig. 4.

range of 1.25–62.5 nM. In the presence of 1 μM LY, the stimulatory effect of 12.5 nM TPA was reduced from 253.04 ± 18.97 to 130.71 ± 5.84 ng GtH/well, while that of 62.5 nM TPA decreased from 323.86 ± 8.08 to 175.25 ± 13.22 ng GtH/well (mean ± SEM; $n = 4$).

The peak of GtH release in response to a 5 min pulse of 0.05 mM AA was similar in the absence or presence of 1 μM DA and no significant rebound above the basal release could be noted after DA removal (Fig. 5). In static culture too, where the cells were exposed to AA (1–100 μM) for 3 h, none of the DA agonists (APO, LY or BRCR, 1 μM) had any inhibitory effect on GtH release (Fig. 6).

The involvement of Ca^{2+} influx in the release of GtH

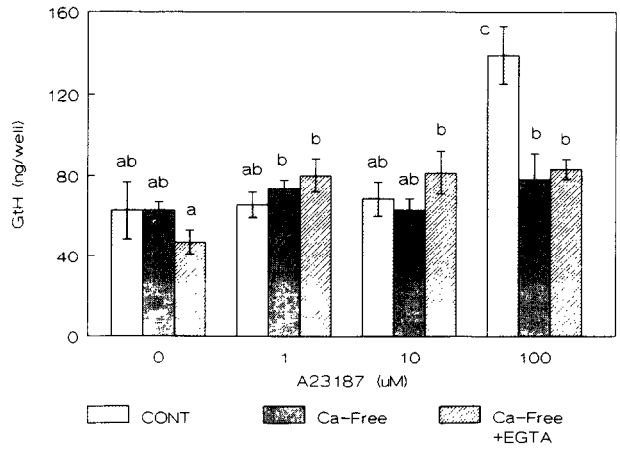

Fig. 7. GtH release from primary culture of dispersed pituitary cells in response to graded doses of A23187. The stimulation medium contained buffered physiological saline (BPS) The control wells (CONT) were exposed to the ionophore in BPS only; parallel wells were exposed to the ionophore in Ca^{2+}-free BPS (Ca-Free) to which 1.2 mM NaCl was added. Another group of wells was exposed to the ionophore in Ca^{2+}-free BPS with 0.1 mM EGTA (Ca-Free + EGTA). The statistical analysis is as in Fig. 4.

was studied in cultured pituitary cells exposed to the Ca^{2+} ionophore A23187 in normal medium or in Ca^{2+}-free medium with or without EGTA (0.1 mM). The stimulatory effect of the ionophore was only evident in the presence of extracellular Ca^{2+} (Fig. 7). Cultured pituitary cells were exposed to graded doses of the ionophore A23187 in the presence of 1 μM of the DA agonists. The Ca^{2+} ionophore stimulated GtH release only at a concentration of 100 μM. At this concentration, the stimulatory effect of the ionophore was abolished by all DA agonists tested (Fig. 8).

In the presence of Ca^{2+}, ionomycin (0.1–10 μM) stimulated GtH release from cultured pituitary cells in relation to the dose. The absence of Ca^{2+} with or without EGTA resulted in a significant but small reduction of the ionomycin-stimulated GtH release (Fig. 9). Addition of the DA agonists did not result in any significant reduction of ionomycin-stimulated GtH release (Fig. 10). In perifu-

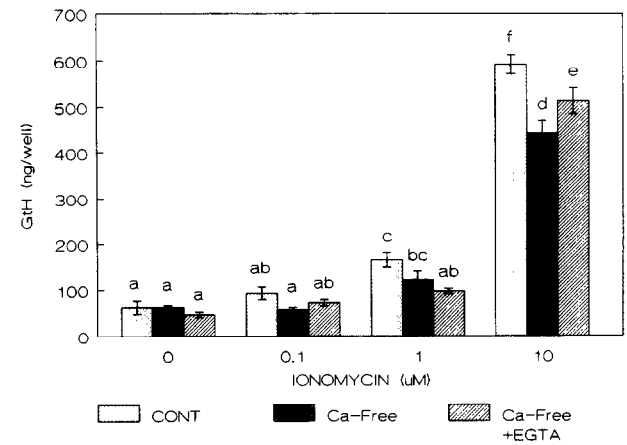

Fig. 9. GtH release from primary culture of dispersed pituitary cells in response to graded doses of ionomycin given in BPS (CONT), Ca^{2+}-free BPS (Ca-Free) or Ca^{2+}-free BPS with EGTA (Ca-Free + EGTA). Other details are as in Fig. 7.

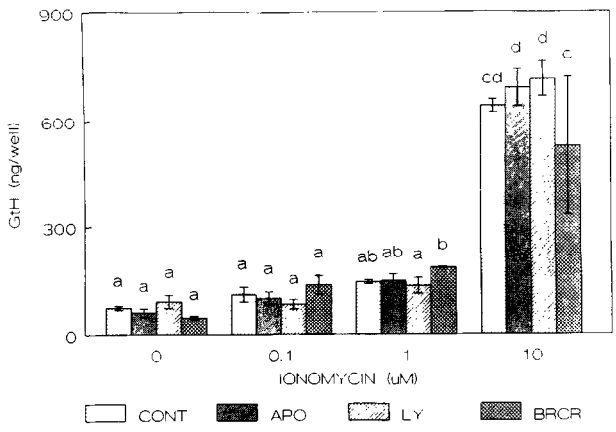

Fig. 10. GtH release from primary culture of dispersed pituitary cells in response to graded doses of ionomycin in the absence (CONT) or presence of 1 μM of APO, LY or BRCR. Other details are as in Fig. 4.

sion of pituitary fragments too, the stimulation by ionomycin (10 μM) was not altered in the presence of 1 μM DA (Fig. 11).

In order to ascertain that GtH release in response to ionomycin or AA is specific and is not due to leakage through disrupted plasma membrane, LDH activity was determined in the medium of cultured cells exposed to these compounds (10 μM and 100 μM, respectively). LDH activity in the medium was found to be less than 0.3% of the activity determined in the cytosol of the cultured cells compared with 1.3% in the medium of cells stimulated by sGnRH.

4. Discussion

The extent of spontaneous or stimulated GtH release in culture varied in its magnitude among experiments. These variations can be attributed to seasonal changes in the reproductive state of the donor fish as has been reported in the trout where such seasonal variations were shown to depend on gonadal steroids (Weil and Marcuzzi, 1990a,b). However, the patterns of the response to the various secretagogues and drugs, compared with the respective controls, were consistent throughout the study.

The maximal stimulatory effects of sGnRH, cGnRH-I and cGnRH-II were not significantly different, however, sGnRH and cGnRH-II were more potent in releasing GtH (with ED_{50} values in the pM range) than cGnRH-I (with ED_{50} values in the nM range). Nevertheless, the stimulatory effect of all GnRH forms tested was abolished by apomorphine. The present study corroborates earlier findings in vivo (Gissis et al., 1991) and in vitro (Yaron and Levavi-Sivan, 1991) that tilapia falls within the category of teleost fish having clear dopaminergic inhibition of GtH release. Dopamine at 10 or 100 μM can inhibit GnRH release from neurons in the hypothalamus and the pituitary of the goldfish (Yu et al., 1991). The results of the present experiments (Fig. 1) conducted on dispersed pituitary cells, in which the contact with nerve fibres is disrupted, indicate that the dopaminergic inhibition of GtH release is exerted directly on the pituitary cells.

From studies in the goldfish and catfish, using specific dopamine agonists and antagonists, it was concluded that dopaminergic inhibition of GtH release is conveyed

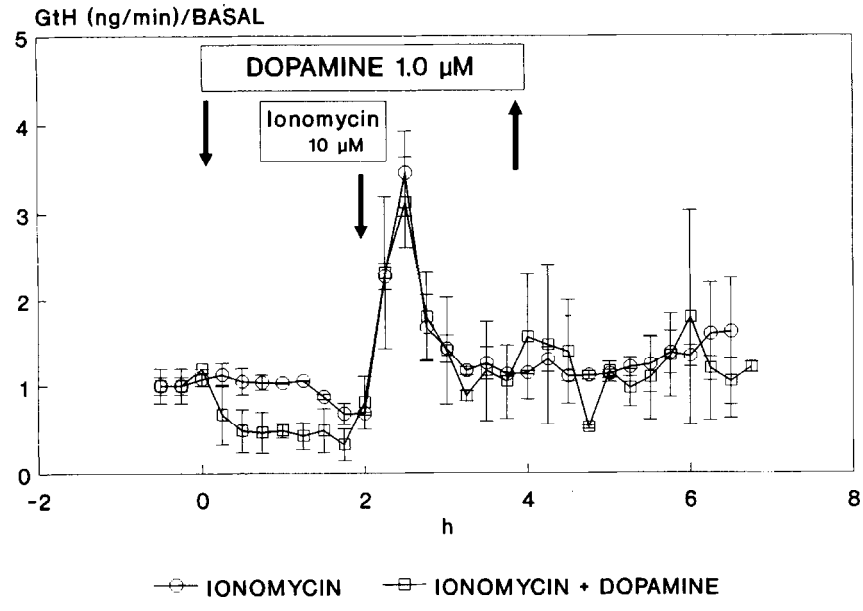

Fig. 11. GtH release in the effluent medium of perifused tilapia pituitary fragments in response to ionomycin in the presence or absence of 1 μM DA. Other details are as in Fig. 2.

through D_2 type receptors (Omeljaniuk and Peter, 1989; Van Asselt et al., 1990; Chang et al., 1991b). Studies on the action mechanism of D_2 type receptors in other systems, such as the rat lactotrophs (e.g. Swennen and Denef, 1982) have shown that DA causes a decrease in cellular cAMP correlated with a reduced prolactin secretion. Also in the frog neurointermediate lobe, DA reduces cAMP production in response to forskolin (Desrues et al., 1993). These and other reports in mammals have led to the conclusion that activation of D_2 type receptor is coupled with the inhibition of adenylate cyclase and a rapid decrease of cAMP levels (reviewed by Vallar and Meldolesi, 1989).

Previous studies in this laboratory have shown that forskolin, dbcAMP and cholen toxin stimulate GtH secretion from perifused tilapia pituitary fragments and cultured cells. Moreover, exposure of the fragments or cells to GnRHa stimulated cAMP formation in correlation with GtH release (Levavi-Sivan and Yaron, 1991, 1992). In the current study, the presence of DA in the perifusion medium totally abolished the GtH surge in response to GnRHa but did not diminish the surge in cAMP formation (Fig. 2). The forskolin-stimulated GtH release from the fragments was also abolished by DA without any effect on cAMP (Fig. 3). A previous study showed that DA curtailed GtH release from perifused pituitary in response to 3 mM dbcAMP (Yaron and Levavi-Sivan, 1991). In the goldfish too, APO reduced the stimulatory effect of forskolin and 8-bromo-cAMP on GtH release from dispersed pituitary cells (Chang et al., 1992). The present results, together with the inability of dbcAMP to prevent dopaminergic inhibition of GtH release, are consistent with the hypothesis that this inhibition in tilapia is exerted at a site or sites distal to cAMP formation. Such a mode of action differs from the classic characteristic of D_2 receptors (reviewed by Niznik, 1987; Vallar and Meldolesi, 1989). Diversion from the classic characteristic of D_2 type receptor was noted also in the goldfish where the analysis of the inhibitory effect of two isomers of apomorphine on GtH and α-MSH release showed only a weak stereoselectivity (Omeljaniuk et al., 1989).

DA agonists (APO, LY and BRCR) attenuated GtH release from cultured pituitary cells in response to DiC8 (Fig. 4) and LY reduced TPA-stimulated GtH release. These results corroborate a previous study in tilapia demonstrating that DA inhibits the release of GtH from perifused pituitary fragments in response to OAG (Yaron and Levavi-Sivan, 1991) and in the goldfish where LY reduces TPA or DiC8-stimulated GtH release in static culture (Jobin and Chang, 1993). The above results obtained in fish gonadotrophs resemble the situation in the rat in which pre-incubation with DA inhibited prolactin release in response to TPA (Delbeke and Dannies, 1985). The fact that DA inhibition of GtH release was not reversed by activation of PKC would indicate a site of DA action distal to PKC.

AA was found to be involved in the release of GtH in tilapia (Levavi-Sivan and Yaron, 1993; Figs. 5 and 6) and goldfish (Chang et al., 1989; Chang and Jobin, 1991). Stimulation by AA was not affected by DA in perifusion (Fig. 5) and by DA agonists (APO, LY and BRCR) in cultured cells (Fig. 6). In the goldfish too, stimulation of GtH release by AA was not inhibited by APO (Chang and Jobin, 1991).

Based on the efflux of 2-[^{3}H]deoxyglucose from sheep pituitary cells exposed to AA, it has been concluded that LH release from these cells is due to the detergent-like action of the fatty acid on the plasma membrane (Kaye et al., 1992). However, in cultured pituitary cells of tilapia, AA did not cause any leakage of LDH into the medium, which indicates an integrity of the plasma membranes. It may be assumed, therefore, that dopaminergic inhibition of GtH release in these fish is exerted also at a step preceding the formation of AA. A similar site of DA inhibition was reported in rat lactotrophs in which DA reduces the stimulation of intracellular arachidonate release produced by two prolactin-stimulating peptides, angiotensin-II and TRH (Canonico, 1989). It is also possible that the stimulation of GtH release by AA is conveyed through other pathways.

The Ca^{2+} ionophore A23187 was found to stimulate GtH release from perifused tilapia pituitary fragments (Levavi-Sivan and Yaron, 1989), and from pituitary cells in culture (Levavi-Sivan and Yaron, 1993) but only in the presence of Ca^{2+} (see also Fig. 7). Exposure of the pituitary fragments to DA (1 μM) totally abolished the response to the ionophore (Yaron and Levavi-Sivan, 1991). Moreover, A23187-induced GtH release was inhibited by APO and the D_2 agonists BRCR and LY (Fig. 8). This would indicate an additional site of DA effect distal to Ca^{2+} influx. A similar situation was reported in rat lactotrophs in which A23187-stimulated prolactin secretion was abolished by DA or bromocryptine. The uptake of ^{45}Ca in primary culture of rat pituitary cells in the presence or absence of A23187 was not affected by bromocryptine, indicating that dopaminergic inhibition of prolactin release occurs at a step distal to Ca^{2+} uptake (Tam and Dannies, 1980; Delbeke and Dannies, 1985). It should be noted, however, that in the goldfish, dopaminergic inhibition of GtH release can be reversed by A23187 (Chang et al., 1993).

The effect of ionomycin on GtH release in the present study differed from that of A23187. Ionomycin could stimulate GtH release from cultured pituitary cells in the absence of Ca^{2+} and even in the presence of EGTA (Fig. 9). In the goldfish too, ionomycin at $\geq$10 μM was found to stimulate GtH release even in Ca^{2+}-free medium (Chang et al., 1990). This would indicate that ionomycin can stimulate Ca^{2+} mobilization from intracellular sources in the fish, as reported in the rat (Naor et al., 1988; Naor, 1990). It should be noted, however, that GtH release stimulated by ionomycin was about 15–25% lower in the

absence of Ca^{2+} and in the presence of EGTA (Fig. 10). This would indicate that ionomycin also affects Ca^{2+} influx, but this component does not exceed 25% in this cell preparation.

Another difference between the action of these ionophores was found in regard to dopaminergic inhibition. DA or its agonists inhibited the stimulatory effect of A23187 but not that of ionomycin (Figs. 8, 10, 11). It would appear, therefore, that DA exerts an inhibitory effect on GtH release in tilapia at site(s) distal to Ca^{2+} influx, but proximal to the mobilization of the ion from intracellular sources. Also in clonal pituitary cells of the rat, TRH induces a spike in cytosolic-free Ca^{2+} caused by the release of an ionomycin-sensitive pool of intracellular Ca^{2+} (Albert and Tashjian, 1984). Similarly, dopamine-induced inhibition of prolactin release from rat pituitary lactotrophs could be prevented by ionomycin (Vallar et al., 1988).

The fact that 10 μM ionomycin did not cause any elevation of LDH activity in the medium indicates that the effect of ionomycin should not be attributed to a non-specific effect caused by disruption of the cellular membrane. Furthermore, at the same concentration, ionomycin did not cause any increase in the release of growth hormone from cultured pituitary cells of this fish (Melamed, unpublished results).

In summary, the present study indicates that dopaminergic inhibition of GtH release from the pituitary of tilapia can be traced along several signal transduction steps. In contrast to the classic characteristic of the DA D_2 receptor mode of action, DA does not reduce cAMP production in response to GnRH, and probably exerts its effect at a step distal to the formation of the nucleotide. DA possibly inhibits Ca^{2+} mobilization from intracellular sources; under such an inhibition, GtH release can be stimulated by ionomycin but not by A23187.

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