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GABA-ergic control of prolactin release in rainbow trout (*Oncorhynchus mykiss*) pituitaries *in vitro*

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Keywords: prolactin, γ-aminobutyric acid, perifusion, primary culture, rainbow trout

Abstract

The involvement of γ-aminobutyric acid (GABA) in the control of prolactin (PRL) release was investigated in rainbow trout using both perifused pituitary fragments and pituitary cells in primary culture. In our perifusion system, infusion of GABA (10^{-6} to 10^{-4} M) caused an inhibition of PRL release (between 20 and 40%). Administration on perifused pituitary fragments of 3APS, a GABAa agonist, mimicked this inhibitory effect. Moreover, bicuculline, a specific antagonist of GABAa receptors, totally abolished GABA effect. When tested on cultured pituitary cells during 40h exposure, GABA (10^{-5} M) caused a significant decrease in PRL release (24.5%). Baclofen, a specific agonist for GABAb receptor tested at 10^{-6} and 10^{-5} M, also inhibited PRL released from cultured pituitary cells. These results demonstrate that GABA inhibits PRL release by acting directly on pituitary cells and that probably both types of GABA receptor (a and b) are involved in this regulation.

Résumé

Nous avons étudié l'implication de l'acide γ-aminobutirique (GABA) dans le controle de la sécrétion de prolactine (PRL) chez la truite arc-en-ciel en utilisant à la fois des fragments d'hypophyse perifusés et des cellules hypophysaires en culture. Dans notre système de périfusion, le GABA (10^{-6} à 10^{-4} M) inhibe la libération de PRL (entre 20 et 40%). L'administration sur les fragments d'hypophyse périfusés de 3APS, un agoniste des récepteurs GABAa, reproduit ces effets inhibiteurs. De plus, la bicuculline, un antagoniste spécifique des récepteurs de type GABAa, abolie complètement les effets du GABA. Lorsqu'il est testé pendant 40h sur des cellules en culture, le GABA (10^{-5} M) réduit de manière significative la libération de PRL (24.5%). Le Baclofen, un agoniste spécifique des récepteurs GABAb testé à 10^{-6} et 10^{-5} M, inhibe aussi la libération de PRL par les cellules en culture. Ces résultats démontrent que le GABA inhibe la libération de PRL en agissant directement sur les cellules hypophysaires et que les 2 types de récepteurs GABA (a et b) sont impliqués dans cette régulation.

Introduction

In mammals, contradictory findings have been reported regarding the control of pituitary functions by γ-aminobutyric acid (GABA). The presence of two different GABAergic systems in the mediobasal hypothalamus is associated with a dual action of GABA (inhibitory and stimulatory) on

prolactin (PRL) release (Apud *et al.* 1989). GABA has been shown to inhibit the secretory activity of PRL cells by acting directly at the levels of anterior pituitary (Enjalbert *et al.* 1979; Locatelli *et al.* 1979). Moreover, at the level of hypothalamus, GABA modulates the activity of the tubero-infundibular dopaminergic system, thus leading to an increase of PRL release (Racagni *et al.* 1982; McCann *et al.* 1984).

In teleost fish, little attention has been paid to the control of anterior pituitary functions by GABA. In the goldfish pituitary, radioautographic and immunocytochemical studies demonstrated the presence of a dense GABAergic innervation of both anterior and neurointermediate lobes (Kah *et al.* 1987b). Moreover, Kah *et al.* (1987a) provided evidence for direct synaptic contacts between GABA endings and secretory cells. An innervation of pituitary by GABA fibres was also observed in carp (Follenius 1972) and in rainbow trout (Dubourg, P., Gonnet, F. and Kah, O., unpublished data). However, *in vitro* studies of the control of PRL release by GABA did not show any effect of this neurotransmitter either in tilapia, *Sarotherodon mossambicus* (Wigham *et al.* 1977), or in rainbow trout (James and Wigham 1984). In the latter, measurements of PRL levels were performed using an electrophoretic technique which was not demonstrated to be specific for PRL and therefore, results should be interpreted with caution.

In order to bring physiological data which would provide significance to GABAergic innervation of PRL cells in rainbow trout, we undertook to redetermine whether GABA modifies PRL secretion *in vitro*. This study was performed using two techniques developed for trout in our laboratory: *in vitro* perifusion of pituitary fragments and primary culture of pituitary cells.

Materials and methods

Experimental animals

Immature rainbow trout (*Oncorhynchus mykiss*) of both sexes were purchased from a freshwater hatchery and kept in recirculating tap water at 13°C under natural photoperiod.

Perifusion experiments

The animals were killed between 15:00 and 16:00h and the pars distalis was dissected under the microscope. The pituitary fragments thus obtained were preincubated for 10 min in culture medium (RPMI—medium-GIBCO/BRL—supplemented with HEPES 20 mM, $NaHCO_3$ 9 mM, penicillin 100 U/ml, fongizone 0.25 g/ml and bovine serum albumin 0.3%—fraction V, SIGMA—). The pH and the osmotic pressure of this medium were adjusted to pH 7.4 and 300 mOsm, respectively. The pituitary fragments were placed in the perfusion system (Gonnet *et al.* 1988) and perfused with the medium described above at low flow rate (3.6 ml/h) for 16h at 15°C. The following day, flow rate was progressively increased to 25 ml/h. The system was equilibrated for 2h before effluent medium was collected as 5 min fractions during the stabilization period and as 0.75 or 2.5 min fractions during infusion of the various secretagogues. The fractions collected were frozen at −20°C until assay.

Secretagogues

GABA, 3-amino-1 propane sulfonic acid (3APS), bicuculline and aminooxyacetic acid were purchased from Sigma Chemical Company.

Primary culture of pituitary cells

The procedures for preparing dispersed and cultured cells from rainbow trout pituitary glands have already been described in details by Weil *et al.* (1986) and Le Goff *et al.* (1992). In these series of culture experiments, pre-treatment with poly-L-lysine (Sigma Chemical Company, 5 g/cm^2) was applied to culture plates before cell plating.

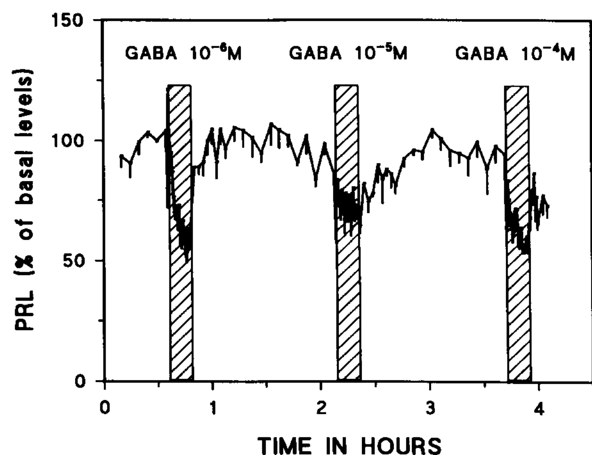

Fig. 1. Effect of increasing concentrations of GABA on PRL secretion by perifused pituitary fragments. After a 16h equilibration period GABA was infused for 15 min. Data represent the mean ± SEM of four independent perifusion experiments. The reference level of PRL release (100%) was calculated for each experiment as the mean PRL secretion rate for 30 min just preceding infusion of the first dose of GABA.

PRL radioimmunoassay

PRL secreted from perifused pituitary fragments or from cultured pituitary cells was determined using a PRL salmon RIA according to the technique described by Prunet *et al.* (1985). This RIA has been demonstrated to be specific for measurement of PRL in rainbow trout (Prunet *et al.* 1985).

Calculation

The perifusion profiles were calculated and expressed as percentages of basal secretory level. The basal level (indicated as 100%) was calculated as the mean of three fractions just before the infusion of secretagogues. Each figure represents the mean profile established from four independent experiments. In the primary culture experiments, PRL measurements were subjected to Mann-Witney non-parametric test for comparing the differences between group means. Differences between groups were considered significant if $p < 0.05$.

Results

Effect of GABA on PRL secretion

Infusion of different doses of GABA (10^{-7} to 10^{-5} M) on perifused pars distalis which were equilibrated for 90 min did not lead to consistent changes in PRL secretion (data not shown). However, after an overnight equilibration period, infusion of GABA (10^{-6} to 10^{-4} M) for 20 min inhibited PRL release (Fig. 1). This inhibition (between 20% and 40%) occurred just after the onset of GABA administration and the effect was sustained during the rest of the infusion. Variability in the levels of basal PRL secretion observed during perifusion did not allow us to obtain a clear dose-related effect of GABA on PRL secretion.

Effect of GABA on PRL released from pituitary cells in primary culture was also studied. Amino-oxyacetic acid (AOAA), a GABA-transaminase inhibitor, was added in the culture medium at the dose of 2.4 10^{-6} M in order to protect GABA from degradation. When cultured pituitary cells were exposed to GABA during 40h, a significant decrease (24.5%) was observed with the dose 10^{-5} M (Fig. 2). GABA at 10^{-6} M induced a slight but non-significant decrease in PRL release. When GABA was tested for a shorter period of time (6, 12 or 24h), no significant effect on PRL secretion was observed (data not shown).

Effect of GABA agonists and antagonist on PRL secretion

In order to test the specificity of these GABA effects, different agonists or antagonists of the two class of GABA receptors (classified as GABAa and GABAb) were tested on PRL secretion. Administration to perfused pituitary fragments of 3APS (10^{-5} M), a GABAa agonist (Nistri and Constani 1979), mimicked the inhibitory effect of GABA (Fig. 3). As shown in Fig. 4, bicuculline (10^{-4} M), a specific antagonist of GABAa receptors, totally abolished the inhibitory effect of infused GABA (Fig. 4). Addition of GABA alone served as internal control. Interestingly, infusion of bicuculline

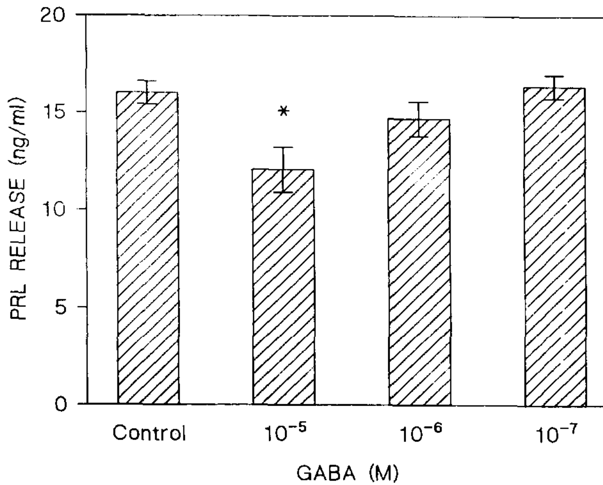

Fig. 2. PRL release from pituitary cells in primary culture after treatment with various concentrations of GABA. Six × 10^4 cells/well were initially plated. The treatment was performed 3 days after plating, for 40h. Values are means ± SEM (n = 5). *p < 0.05 compared to control.

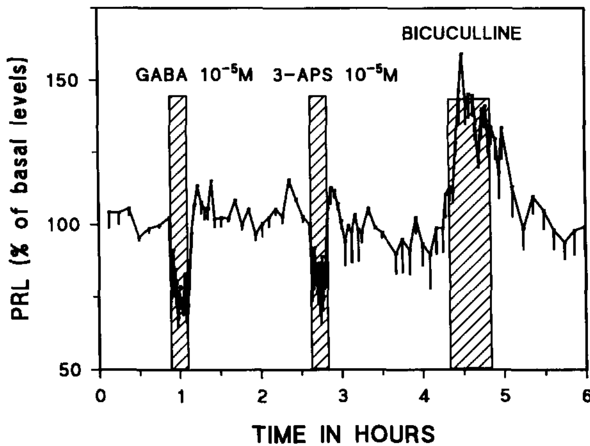

Fig. 3. Effect of GABA (10^{-5} M), 3APS (10^{-5} M) and bicuculline (10^{-4} M) on PRL secretion by perifused rainbow trout pituitaries. After 16h equilibration period, GABA and later 3APS were perfused for 20 min, whereas bicuculline was perfused for 30 min. Data were treated as for Figure 1.

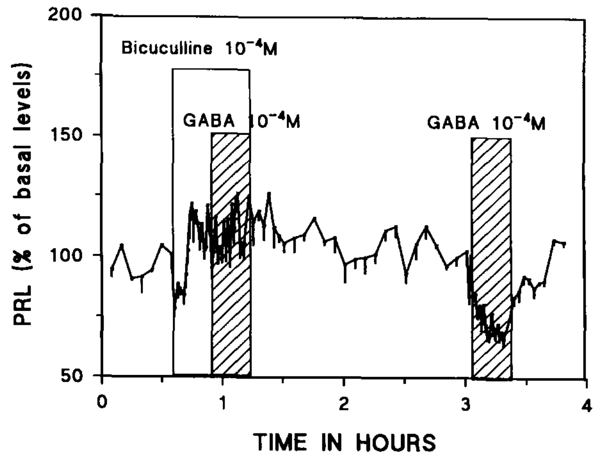

Fig. 4. Effect of GABA (10^{-4} M) in the presence or absence of bicuculline (10^{-4} M) on PRL secretion by perifused rainbow trout pituitaries. After a 16h equilibration period, bicuculline was perfused for 40 min. Twenty min after the onset of bicuculline administration, GABA was infused for 20 min in the presence of bicuculline. Data were treated as for Figure 1.

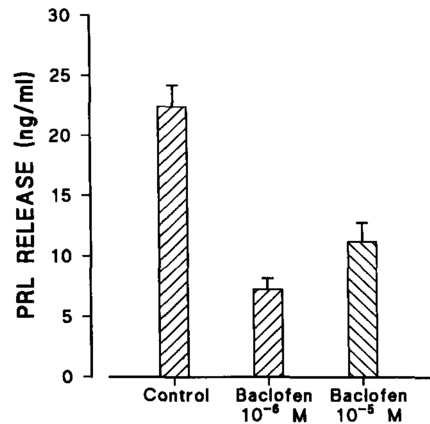

Fig. 5. PRL release after treatment with two different concentrations of baclofen, a GABAb agonist. Six × 10^4 cells/well were initially plated. The treatment was performed 3 days after plating for 40h. Values are means ± SEM (n = 5). **p < 0.001 compared to control.

(10^{-4} M) alone induced an increase of PRL secretion (Fig. 3).

Baclofen, a specific agonist for GABAb receptors, was also tested. When administered to perifused pituitary fragments, baclofen did not show any consistent effects (data not shown). However, when incubated for 40h with cultured pituitary cells at concentrations of 10^{-5} or 10^{-6} M, this GABAb agonist significantly inhibited PRL secretion (Fig. 5).

Discussion

In the present study, we provide evidence that GABA is able to inhibit PRL secretion from rain-

bow trout pituitary *in vitro*. Moreover, GABA is also able to act directly at the level of the PRL cells, as indicated by the inhibitory effects of GABA on PRL released from cultured pituitary cells. Thus, these results provide physiological significance for the widespread GABAergic innervation described in the goldfish and rainbow trout pituitary, close to the PRL cells (Kah *et al.* 1987a; Dubourg, P., Gonnet, F. and Kah, O., unpublished data). These results are in agreement with numerous studies in the rat pituitary showing a direct inhibitory control of PRL secretion by GABA (Enjalbert *et al.* 1979; Locatelli *et al.* 1979; Racagni *et al.* 1982; Loeffler *et al.* 1986).

In our perifusion system, effective doses of GABA which inhibited PRL secretion range between 10^{-6} and 10^{-4} M. Similar ranges of doses were also used in studies on the effect of GABA on MSH secretion using superfused amphibian intermediate lobes (Adjeroud *et al.* 1986; Verburg-van Kemenade *et al.* 1987). Moreover, GABA inhibited PRL released from rat incubated hemipituitaries using doses between 10^{-6} and 10^{-5} M (Enjalbert *et al.* 1979). Interestingly, in our perifusion experiments, infusion of bicuculline, a specific antagonist for GABAa receptor, induced an increase of the basal PRL release. A similar result was observed by Adjeroud *et al.* (1986) on perifused frog intermediate lobes. This suggests the presence of an endogenous GABA-ergic inhibitory tonus in the perifused tissue. In the present study, such hypothesis is further supported by absence of clear GABA effect when perifused pituitary fragments were only equilibrated for 2h and the necessity of an overnight equilibration period which probably eliminates part of this GABA-ergic tonus. In this context, one would expect that only high doses of GABA would inhibit PRL secretion. Moreover, possibility of metabolization of GABA by GABA-transaminase during our perifusion experiments may also explain why it is necessary to use these doses. In mammals, a high GABA-transaminase activity was localized in the pituitary which was devoid of GAD activity, enzyme responsible for GABA synthesis (Racagni *et al.* 1979). Finally, in absence of reported values for GABA concentrations which reach PRL cells in fish, it is difficult to conclude whether the doses used in the present study are physiological or not.

More unexpected were our results showing that only a large dose (10^{-5} M) of GABA added for 40h was able to inhibit PRL release in cultured pituitary cells. This effect could only be observed after addition of AOAA, an inhibitor of GABA-transaminase, which would protect GABA from degradation (Duvilanski *et al.* 1985). Moreover, baclofen, a GABAb agonist, incubated in similar conditions appeared to be more potent than GABA as 10^{-6} M dose induced 50% inhibition of PRL release. This suggests a possible partial degradation of GABA during incubation. Shorter periods of exposure to GABA were tested without leading to significant effect on PRL release. Such delay in the response of trout PRL cells in primary culture has already been described when studying the effects of somatostatin (Le Goff *et al.* 1992). This situation, not observed with GH cells, was suggested by Le Goff *et al.* (1992) to be associated with a particular behaviour of cultured PRL cells which seem to be under a dominant stimulatory control by hypothalamus in rainbow trout (Gonnet *et al.* 1989; Yada *et al.* 1991).

The use of high flow rates and short collection time in our perifusion experiments have led to a clear description of the PRL response to GABA infusion. Our results show an immediate inhibitory effect of GABA on PRL release and this effect disappeared when GABA was removed. Similar results were reported on α-MSH release in the toad, *Xenopus laevis*, where GABA only induced inhibition on either intact intermediate lobes or dispersed pars intermedia cells (Verburg-van Kemenade *et al.* 1986, 1987). However, GABA was also shown to have biphasic effects on hormone release: in the frog and in the rat, GABA stimulates release for several minutes before inhibiting the secretory process (Tomiko *et al.* 1983; Tonon *et al.* 1986). Thus, GABA actions on PRL release in rainbow trout seem to be similar to what was described in the toad where Verburg-van Kemenade *et al.* (1987) concluded that this inhibitory action was mediated mainly by GABAb receptors. Whereas we were unable to observe any significant effect of baclofen, a GABAb receptor agonist, on PRL secreted by peri-

fused pituitary fragments, this agonist had strong inhibitory effect on PRL cells in primary culture. This suggests the possible involvement of GABAb receptors in the inhibitory action of GABA. GABAa receptors appeared also to be involved in the action of GABA on perifused pituitary fragments. A GABAa agonist, 3APS, mimicked the inhibitory effect of GABA. This effect of GABA could also be reversed by bicuculline, a specific antagonist of GABAa receptor. Occupancy of GABAa sites has also been suggested to induce inhibition of α-MSH release in a frog (Tonon *et al.* 1989). Although both types of GABA receptors seem to be involved in the inhibition of PRL secretion in rainbow trout, further studies are needed to clarify their precise roles.

In conclusion, the present study demonstrates that GABA acts directly on pituitary cells to inhibit PRL secretion, thus supporting the view that this inhibitory neurotransmitter may be a putative modulator of PRL cell activity in rainbow trout.

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