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Nicotine stimulated GtH2 secretion in vivo in male common carp (*Cyprinus carpio*); Potentiation of GnRH action and possible interaction with dopaminergic system

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Abstract – Nicotine injected intravenously or on pituitary in vivo stimulated maturational gonadotropin (GtH2) secretion in domperidone (D-2 receptor antagonist) pretreated carps. The effective dose was 1 $mg \cdot kg^{-1}$ and 100 $\mu g \cdot kg^{-1}$, respectively. No stimulatory effects of nicotine were observed in normal (non-treated) carps. When nicotine was injected together with gonadotropin releasing hormone (GnRH) analogs (D-Arg^6 sGnRH or D-Ala^6 LHRH) a clear potentiation effect of nicotine on GnRH analogs' action was observed. This potentiation was seen even in normal fish where nicotine itself had no effect on GtH2 release. A synergism between nicotine and GnRH analogs appeared when low doses of GnRH analogs were used. In domperidone pretreated fish, this synergism occurred after injection of 1 $\mu g \cdot kg^{-1}$ of sGnRHa. In normal fish, the same phenomenon was observed after injection of 5 $\mu g \cdot kg^{-1}$ of sGnRHa or 20 $\mu g \cdot kg^{-1}$ of LHRHa. Higher doses of GnRH analogs mask this effect. Results confirm previous in vitro findings on the stimulatory action of nicotine on GtH2 secretion. They also indicate a possible synergy between nicotine and GnRH as well as an inhibitory influence of dopamine on nicotine's action. © Ifremer/Elsevier, Paris

Sex hormones / sexual maturity / fish physiology / *Cyprinus carpio*

Résumé – Stimulation in vivo par la nicotine de la sécrétion de GtH2 chez la carpe commune (*Cyprinus carpio*) ; potentialisation de l'action de GnRH et interaction possible avec le système dopaminergique. L'injection de nicotine par voie intraveineuse ou directement sur l'hypophyse stimule in vivo la sécrétion de gonadotropine (GtH2) chez la carpe traitée préalablement par un antagoniste des récepteurs D2, la dompéridone ; les doses effectives sont respectivement de 1 $mg \cdot kg^{-1}$ et 100 $\mu g \cdot kg^{-1}$. La nicotine n'a pas d'effet chez les animaux non traités par l'antagoniste D2. Injectée en même temps qu'un analogue de l'hormone (GnRH), le D-Arg^6 sGnRH ou le D-Ala^6 LHRH, elle induit une claire potentialisation de l'action de ces analogues sur la stimulation de la sécrétion de GtH2, même en l'absence de dompéridone, alors que, seule, elle n'a pas d'effet. Les effets synergiques entre la nicotine et les analogues de GnRH n'apparaissent qu'aux faibles doses de GnRH. Chez les poissons traités préalablement à la dompéridone, cet effet de synergisme est constaté après l'injection de 1 $\mu g \cdot kg^{-1}$ de sGnRHa. Chez les poissons normaux, ce phénomène est observé suite à l'injection de 5 $\mu g \cdot kg^{-1}$ de sGnRHa ou 20 $\mu g \cdot kg^{-1}$ de LHRHa. Ces résultats confirment des données antérieures obtenues in vitro. Ils montrent également une synergie possible entre la nicotine et la GnRH dans la libération de GtH2, ainsi qu'une inhibition des effets de la nicotine par la dopamine. © Ifremer/Elsevier, Paris

Hormones sexuelles / maturité sexuelle / physiologie des poissons / *Cyprinus carpio*

1. INTRODUCTION

The special anatomical construction of fish pituitary gland and especially the lack of a hypothalamo-hypophyseal portal system make the fish a very interesting model for neuroendocrine research. There is a direct contact of nerve fibers containing GnRH [3, 17], dopamine [4] and GABA [5, 6] with particular secretory cells of the pituitary gland. These three neurotransmitters together with many others (NPY, serotonin, opioid

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peptides, NE and ovarian steroids) are firmly implicated in the neuroendocrine control of maturational gonadotropin (GtH2) secretion in teleost fish [2, 15, 19]. Recently it was shown that drugs belonging to the cholinergic family also affect the GtH2 secretion in fish. Nicotine was shown as a potent in vitro stimulatory factor of GtH2 release in carp [9, 11] and its mechanism seems to be a receptor mediated process [10]. It was also shown that in vitro stimulatory action of nicotine was inhibited by dopamine [11]. All three reports describe the in vitro effects of nicotine by the use of perifusion and primary culture of isolated carp pituitary cells or perifusion of pituitary fragments. In this paper, we present the in vivo effects of nicotine on GtH2 release in the common carp. Nicotine was applied in a form of intravenous (i.v.) or on pituitary (o.pit) injections. The interactions between nicotine and the two most important regulatory neurotransmitters, GnRH and dopamine, were also investigated.

2. MATERIALS AND METHODS

Experiments were conducted during 3 consecutive spawning seasons (1992-1994) of the common carp (*Cyprinus carpio* L.) under Polish climatic conditions (mid May-mid June). Sexually mature (spermiating) common carp males were used. The age of fish varied from 3 to 5 years and their body weight from 1.5 to 3.2 kg. In each experiment, only fish of the same age were used. They all originated from the fish farm belonging to the laboratory. At least 4 days before each experiment, fish were netted from outdoor earth ponds and placed in flow-through 2 m^3 basins with warm (20 °C) water (1 group per basin, 8 fish per group). Carps were exposed to simulated natural photoperiod. Before any manipulations (weighing, tagging, injections, blood sampling), fish were anesthetized in a solution of 2-phenoxy ethanol (5 mL/10 L water). The volume of tested solution injected intraperitoneally (i.p.) was 0.5 $mL \cdot kg^{-1}$. As to the on pituitary (o.pit.) injections, the volume was 20 $\mu L \cdot kg^{-1}$. The protocol of the experiments as well as the technique of the o.pit injections were developed especially for carp and are described in detail elsewhere [12]. Briefly, the injection needle introduced next to the eyeball followed the route of the *nervus opticus* and than automatically reached the environment nearest to the pituitary gland. Intravenous (i.v.) injections (vol of 200 $\mu L \cdot kg^{-1}$) as well as blood samples (300 µL of volume each time) were done by puncturing the caudal vein with 23 G × 1¼ Luer needle attached to a 1 mL syringe. For blood sampling, heparinized syringes were used. After blood centrifugation, the plasma was frozen at –20 °C until analysis for the carp maturational gonadotropin (GtH2) by ELISA as developed by Kah et al. [7] with some minor modifications. The intra-assay variation (8 repetitions) was 4.3 % and the inter-assay variation (8 repetitions) was 8.6 %.

A total of 14 in vivo experiments were conducted. (-)Nicotine (Sigma), dissolved in a BSS Cortland, was given at doses ranging from 10 ng to 100 $\mu g \cdot kg^{-1}$ for o.pit. injections and from 1 µg to 1 $mg \cdot kg^{-1}$ for i.v. injections. GnRH analogs – D-Arg^6 salmon GnRH (sGnRHa) (Bachem) or D-Ala^6 LHRH (LHRHa) (Sigma) were injected intraperitoneally (i.p.) at doses ranging from 1 to 20 $\mu g \cdot kg^{-1}$. Domperidone (gift from Jenssen Pharmaceuticals) was injected i.p. in a form of suspension in BSS Cortland at a dose of 5 $mg \cdot kg^{-1}$ 12 h before the start of a given experiment. For more details concerning each experiment, see *figure* legends. The significance of differences among the groups were calculated using ANOVA followed by Duncan multiple range test at $P < 0.05$ level of significance.

3. RESULTS

3.1. The effects of nicotine given o.pit. on GtH2 secretion

In domperidone-pretreated fish, nicotine at a dose of 100 $\mu g \cdot kg^{-1}$ evoked a significant increase in GtH2 secretion at 45 and 90 min after injection in comparison to all other groups (with the exception of the group nicotine 10 $\mu g \cdot kg^{-1}$ at the time 45 min) (*figure 1*). Lower doses of nicotine (10 ng–10 $\mu g \cdot kg^{-1}$) had no significant effects on GtH2 release (data not shown). No effect of nicotine on GtH2 secretion was observed (at the same doses) in normal (not injected with domperidone) fish (data not shown).

3.2. The effects of nicotine given i.v. on GtH2 secretion

In fish not treated with domperidone, a wide range of nicotine doses (1 µg–1 $mg \cdot kg^{-1}$) had no effect on GtH2 secretion (data not shown). On the contrary, in carps pretreated with domperidone (*figure 2*), a significant stimulatory effect of nicotine (versus control) at a dose of 1 $mg \cdot kg^{-1}$ was observed at 40 and 60 min post injection time. Also the lowest dose (1 $\mu g \cdot kg^{-1}$) had a significant but very short-lasting stimulatory effect on GtH2 secretion 10 min after injection.

3.3. The effects of nicotine on GnRH analog stimulated GtH2 secretion in normal fish

Nicotine itself, at a dose of 1 $mg \cdot kg^{-1}$ (i.v.), had no effects on GtH2 secretion throughout the whole experiment (*figure 3*A). Also the lower dose of sGnRHa (1 $\mu g \cdot kg^{-1}$) (i.p.) evoked no significant changes in GtH2 release in comparison to the control group. A higher dose of sGnRHa (5 $\mu g \cdot kg^{-1}$) significantly increased GtH2 secretion in comparison to the control at 60 min postinjection and in comparison to four other groups (control, nicotine, sGnRHa1, nicotine +

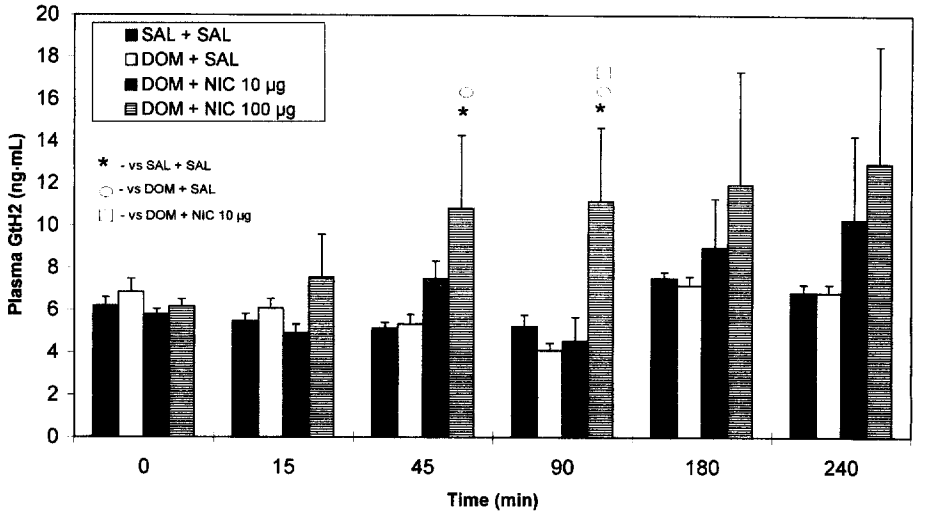

Figure 1. The effect of the on pituitary injections of different doses of nicotine on GtH2 secretion in carps pretreated with domperidone. Domperidone was injected intraperitoneally 12 h before experiment at a dose of 5 mg·kg^{-1}.

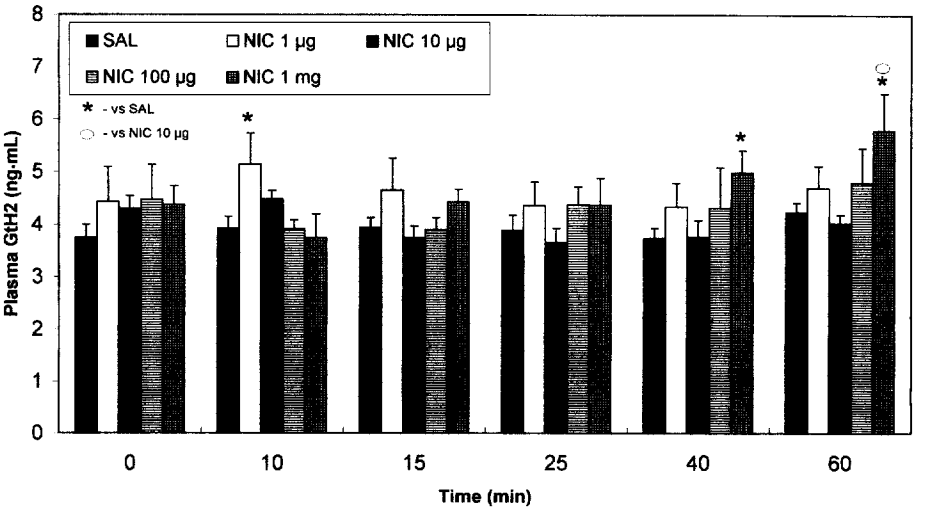

Figure 2. The effect of intravenous injections of different doses of nicotine (from 1 µg to 1 mg·kg^{-1}) on the GtH2 secretion in domperidone pretreated carps. Domperidone (5 mg·kg^{-1}) was injected intraperitoneally 12 h before the experiment.

sGnRHa1) 180 min after injection. The maximum level (12.17 ng·mL of GtH2) was reached in this group. The group injected with a combination of nicotine (1 mg·kg^{-1}) and sGnRHa (5 µg·kg^{-1}) already showed a significant increase of GtH2 level 20 min after injection. At 40 and 60 min after injection, the GtH2 level in this group was significantly higher than in all other groups including sGnRHa 5 µg·kg^{-1} group. At the end of the experiment (time 180 min) there was no longer a difference between sGnRHa and nicotine + sGnRHa groups.

In the experiment where 10 µg·kg^{-1} of sGnRHa was used (*figure 3*B) there was no differences between groups treated with sGnRHa and sGnRHa + nicotine. In both groups, GtH2 levels were significantly higher in comparison to controls starting from 20 min post-injection time, reaching the maximum (20 ng·mL) 60 min after injection. Nicotine itself, like in other experiments, had no effect on GtH2 release.

When the less active GnRH analog - D-Ala^6 LHRH (LHRHa) was used, the highest GtH2 level was reached in the group treated with LHRHa (20 µg·kg^{-1}) together with nicotine (1 mg·kg^{-1}) and was significantly higher from all other groups 60 min after injection (*figure 4*). Nicotine alone (1 mg·kg^{-1}) had no effects on GtH2 secretion. LHRHa alone significantly increased GtH2 secretion starting 40 min after injection up to the end of the experiment (180 min).

3.4. The effects of nicotine on sGnRHa-stimulated GtH2 secretion in domperidone-pretreated fish

In three experiments, 3 doses of sGnRHa were used: 1, 5 and 10 µg·kg^{-1} (*figures 5*, *6* and *7*, respectively). All of them were tested alone and in combination with nicotine at a constant dose of 1 mg·kg^{-1}. In all experiments, nicotine alone significantly increased GtH2 secretion 60 min after injection in comparison to the control group. Carps treated with sGnRH also showed

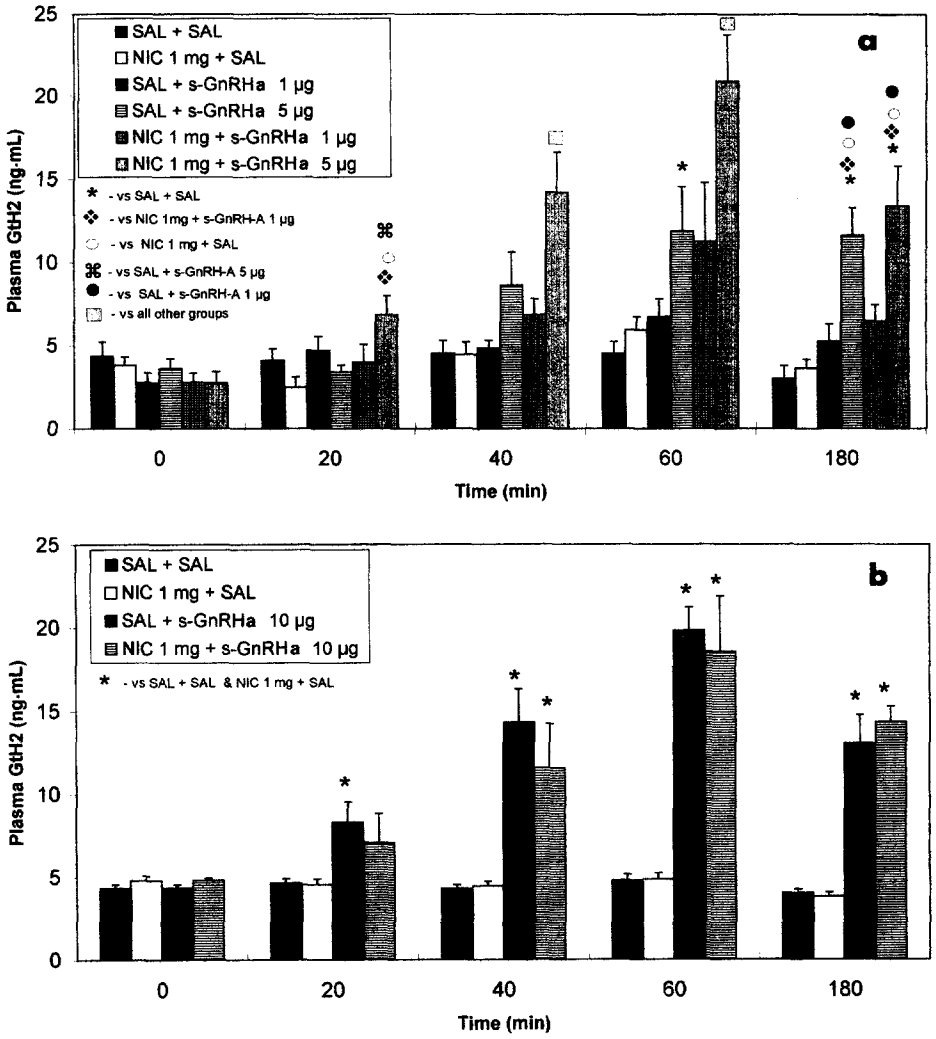

Figure 3. The effects of intraperitoneally injected sGnRHa (1 and 5 $\mu g \cdot kg^{-1}$ – a. 10 $\mu g \cdot kg^{-1}$ – b) alone or in combination with nicotine (1 $mg \cdot kg^{-1}$) given intravenously on GtH2 secretion in the normal (not pretreated with domperidone) carps. sGnRHa and nicotine were administered at the same moment.

a significantly higher GtH2 level compared to control group (1 $\mu g \cdot kg^{-1}$ sGnRHa) and to control group and to nicotine alone (5 and 10 $\mu g \cdot kg^{-1}$ sGnRHa, respectively) reaching the maximum of 13, 28 and 28 ng·mL for doses of 1, 5 and 10 $\mu g \cdot kg^{-1}$, respectively. Combination of the lowest dose of sGnRHa (1 $\mu g \cdot kg^{-1}$) with nicotine evoked significantly higher GtH2 secretion in comparison to all other groups including sGnRHa alone at time 40 and 60 min after treatment. As to the two higher doses of sGnRHa, there was no significant differences between sGnRHa treated groups and groups treated with a combination of sGnRHa and nicotine.

4. DISCUSSION

Direct stimulatory action of nicotine on GtH2 secretion in carp has been recently demonstrated using the in vitro techniques of pituitary cell perifusion and culture [9, 10]. This stimulatory action is exhibited in both sexes and is a dose and receptor dependent process. The results presented in this paper and obtained using in vivo techniques fully confirm previous findings. Moreover, they show a more complex picture of the mechanism of nicotine action upon GtH2 secretion in carp. In all the experiments where normal (not DA antagonist pretreated) fish were used, it was impossible to get any stimulation of GtH2 release even when a wide range of nicotine doses and different techniques were used. Even with on pituitary (o.pit.) injections especially developed for injection of extremely non-specific drugs at very low dosages in the direct environment of the pituitary gland [12], there was no effect of nicotine on GtH2 secretion in normal fish. Quite a different situation was observed in fish injected with domperidone - a D2 receptor antagonist. On pituitary or intravenous injections of certain doses of nicotine (100 µg or 1 $mg \cdot kg^{-1}$ respectively) evoked significant increase in GtH2 secretion about 60 min after injection. Lower doses were ineffective. Similar results were obtained using perifusion of whole pituitary

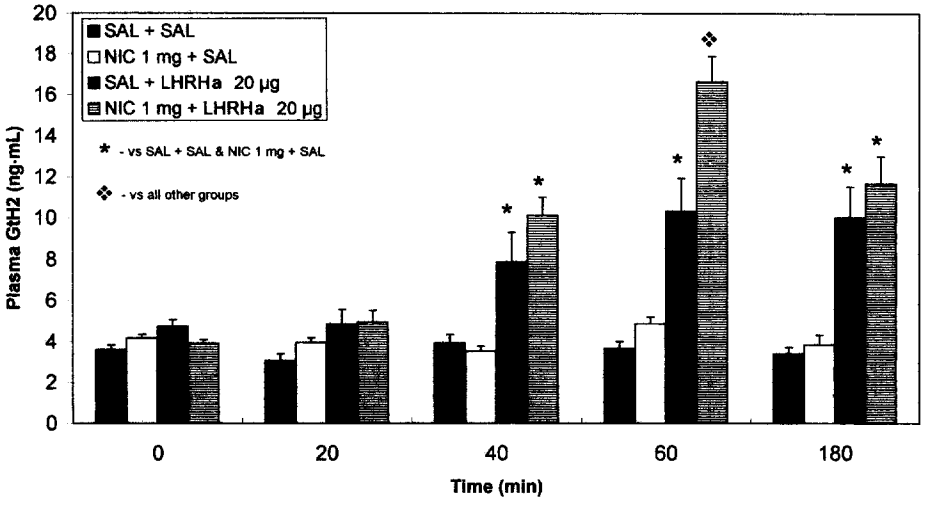

Figure 4. The effect of intraperitoneally injected D-Ala^6 LHRH (20 µg·kg^{-1}) alone or in combination with nicotine (1 mg·kg^{-1}) injected intravenously on GtH2 secretion in normal carps. LHRHa analog and nicotine were injected at the same moment.

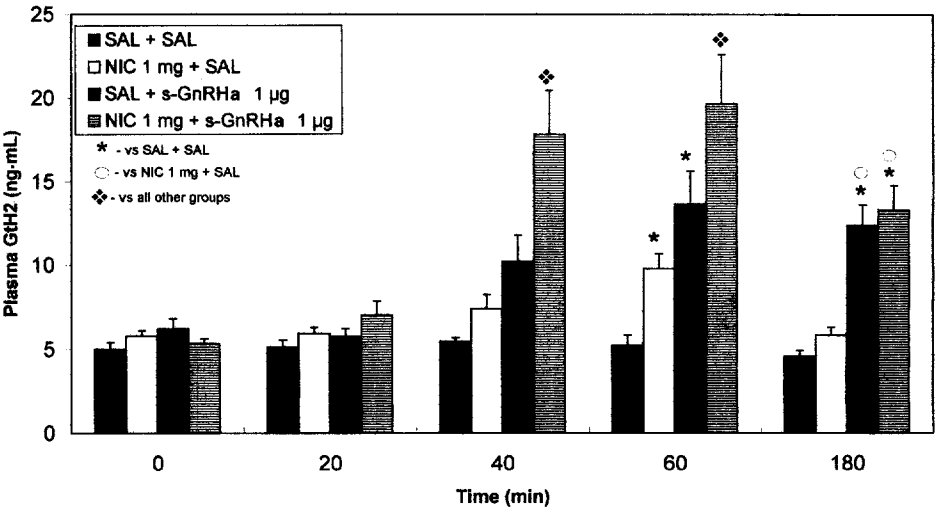

Figure 5. The effect of intravenous injection of nicotine (1 mg·kg^{-1}) and sGnRHa given intraperitoneally at a dose of 1 µg·kg^{-1} on GtH2 secretion in domperidone pretreated carps (5 mg·kg^{-1}, 12 h before experiment). sGnRHa and nicotine were injected at the same moment.

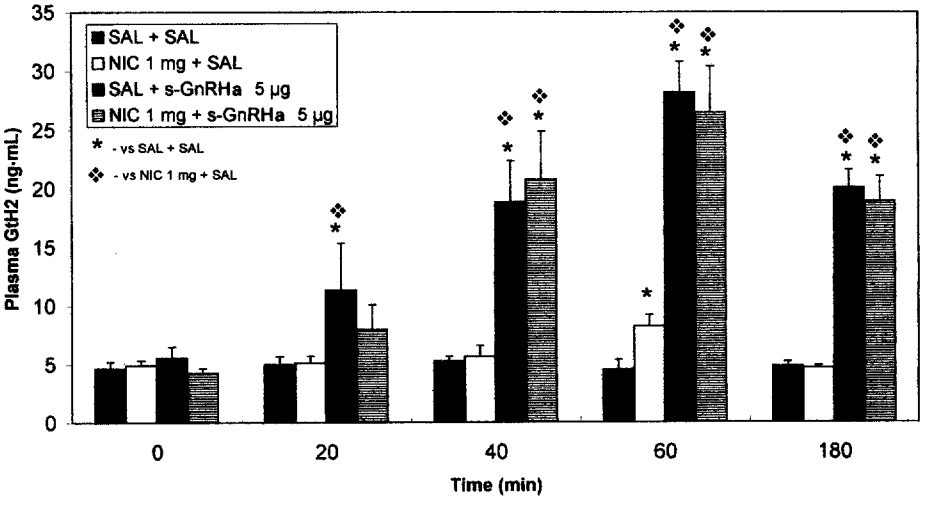

Figure 6. The effect of nicotine and sGnRHa at a dose of 5 µg·kg^{-1} on GtH2 secretion in domperidone pretreated carps. For more details see *figure 5* legend.

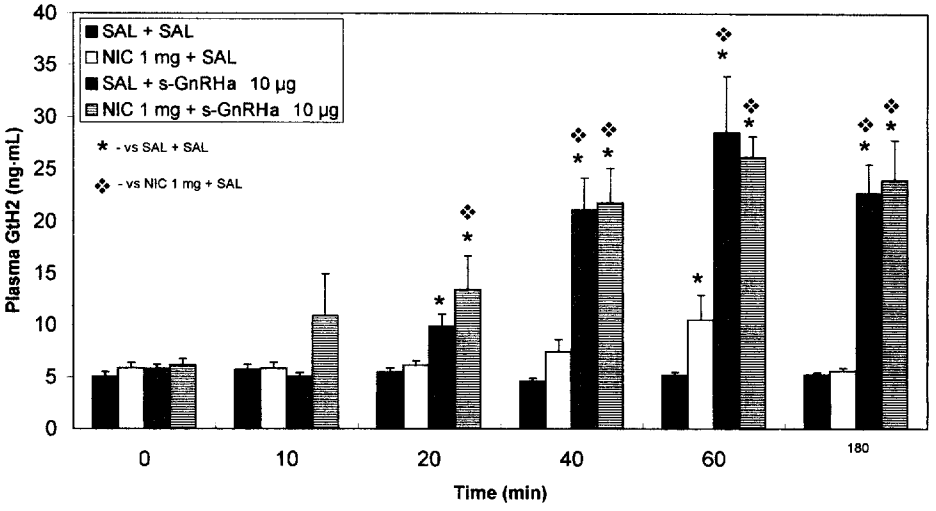

Figure 7. The effect of nicotine and sGnRHa at a dose of 10 $\mu g \cdot kg^{-1}$ on GtH2 secretion in domperidone pretreated carps. For more details see *figure 5* legend.

glands, where the stimulatory action of nicotine was seen if the drug was given in combination with antidopaminergic drugs (haloperidol, reserpine) [11]. These findings tend to indicate that DA plays the important role of inhibitory factor for the stimulatory action of nicotine on GtH2 release, similar to its inhibition of GnRH action [14, 19]. It should be noticed that in vivo stimulatory action of nicotine on GtH2 secretion is much weaker than that observed in vitro using dispersed pituitary cells as a model. In these in vitro studies [9, 10], rather low doses of nicotine (10^{-8} -10^{-6} M) were able to stimulate GtH2 secretion of 250, 300 and even 600 % of the basal secretion level. This difference between in vivo and in vitro nicotine potency can be explained by the fact that, among nicotine's multiple actions, is a well known DA release stimulatory factor in mammalian brain tissue [1, 8, 16, 18]. If a similar phenomenon exists in fish, it is possible to hypothesize that nicotine stimulates GtH2 secretion directly from the pituitary cells and meanwhile, indirectly inhibits its own action by the release of dopamine from neuronal synapses present in the pituitary gland of fish [4].

Experiments, focused on the effects of nicotine on GnRH analog-stimulated GtH2 secretion, confirm once again that nicotine can stimulate GtH2 secretion in domperidone-pretreated fish only. However, the most interesting finding is that nicotine highly potentiates the action of GnRH analogs in domperidone-pretreated as well as in normal fish (where nicotine alone had no effect). In both types, the effect of the joined action of nicotine and GnRH analogs is not additive but seems to be synergistic. From our results, it is clear that the potentiation effect of nicotine on GnRH analogs depends on their dose and potency. The potentiation effect of nicotine was seen when sGnRHa was injected at a dose of 1 $\mu g \cdot kg^{-1}$ in domperidone-pretreated fish and at a dose of 5 $\mu g \cdot kg^{-1}$ in normal fish. When the higher or lower doses of sGnRHa were used, there was either no stimulatory effect or the potentiation effect of nicotine was masked and not seen any more. As to the less active analog D-Ala^6 LHRH [20], the potentiation effect of nicotine was seen at a dose of 20 $\mu g \cdot kg^{-1}$. It is impossible to find scientific data explaining the mechanisms of the potentiation of the GnRH analogs' action. One of the possible hypotheses could be that nicotine stimulates the release of endogenous GnRH in too small amounts to significantly stimulate the GtH2 release in normal fish, i.e., with high dopaminergic tonus, but effective in fish with blocked DA receptors. It is well known that the responsiveness of the fish pituitary to GnRH stimulation changes following antidopaminergic treatment [13]. It was also recently shown (Breton et al., unpubl.) that in carp, the blood sGnRHa levels (after injection) ranging from 0 to ± 90 pg·mL does not change the secretion of GtH2. The stimulation was observed above that level of sGnRHa. This is also a possible explanation of the fact that only certain doses of GnRH analogs can be potentiated by nicotine since the strong stimulatory effect of higher doses of GnRH analogs can mask the potentiation effect of nicotine.

In conclusion, the present paper confirms earlier in vitro findings concerning stimulatory action of nicotine on GtH2 secretion in carp. Moreover, it shows that the in vivo stimulatory action of nicotine seems to be inhibited by dopamine. The potentiation effect of nicotine on GnRH analogs' stimulatory action needs to be studied further in order to find out the mechanism of this phenomenon.

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