

# Acid stress induces a D<sub>1</sub>-like dopamine receptor in pituitary MSH cells of *Oreochromis mossambicus*

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Lamers, Anne E., Petra J. Ter Brugge, Gert Flik, and Sjoerd E. Wendelaar Bonga. Acid stress induces a D<sub>1</sub>-like dopamine receptor in pituitary MSH cells of *Oreochromis mossambicus*. *Am. J. Physiol.* 273 (Regulatory Integrative Comp. Physiol. 42): R387–R392, 1997.—A 7-day exposure of tilapia (*Oreochromis mossambicus*) to water with a pH of 4.5 activates their pituitary melanophore-stimulating hormone (MSH) cells to preferentially release diacetyl  $\alpha$ -MSH as an important corticotrope (13). We here focus on the control of  $\alpha$ -MSH release by dopamine in tilapia exposed to water with low pH ("low-pH tilapia"). The MSH cells of low-pH tilapia showed a decreased sensitivity to inhibitory concentrations ( $10^{-7}$ – $10^{-6}$  M) of dopamine compared with controls. Low concentrations ( $10^{-14}$ – $10^{-8}$  M) of dopamine stimulated the release of  $\alpha$ -MSH in low-pH tilapia but not in controls. Strong pharmacological evidence for a stimulatory dopamine receptor (D<sub>1</sub>-like) was obtained: the D<sub>1</sub>-agonists SKF-38393 and 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (6-chloro APB) had a stimulatory effect on the release of  $\alpha$ -MSH in low-pH tilapia MSH cells but not in controls. The selective D<sub>2</sub>-agonists quinpirole and 2-hydroxy apomorphin inhibited the release of  $\alpha$ -MSH in controls as well as in low-pH tilapia, and there was no difference in the sensitivity of the cells to these agonists. We conclude that only MSH cells of low-pH exposed tilapia exhibit a D<sub>1</sub>-like receptor activity. A comparable D<sub>2</sub>-like receptor activity, as demonstrated by specific D<sub>2</sub>-receptor agonists, is present in both controls and low-pH-adapted fish. The apparent loss of sensitivity of the MSH cells to inhibitory concentrations of dopamine, therefore, must be caused by the activation of the D<sub>1</sub>-like receptors and not by changes in the activity of the D<sub>2</sub>-like receptor proper. Stimulatory concentrations of dopamine not only quantitatively but also qualitatively enhanced the corticotropic activity of the released  $\alpha$ -MSH, as indicated by the elevated ratio of diacetyl and monoacetyl  $\alpha$ -MSH. This effect was mimicked by the D<sub>1</sub>-like agonists SKF-38393 and 6-chloro APB, indicating that the D<sub>1</sub>-like receptor activity is responsible for the enhancement of the di/mono ratio.

D<sub>2</sub>-like receptor; quinpirole; 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide; SKF-38393; 2-hydroxy apomorphin; diacetyl/monoacetyl ratio; tilapia

IN TILAPIA,  $\alpha$ -melanophore-stimulating hormone ( $\alpha$ -MSH) is a corticotrope hormone rather than a melanotrope hormone (13). During adaptation to water with a low pH, the MSH cells in the pituitary of the tilapia *Oreochromis mossambicus* become activated and show hypertrophy (14). We demonstrated that the total immunoreactive  $\alpha$ -MSH in the blood of low pH-adapted fish is higher than in control blood plasma (13). Both thyrotropin-releasing hormone (TRH) and corticotropin-releas-

ing hormone (CRH) stimulate the release of  $\alpha$ -MSH. As a result of exposure of tilapia to water with a low pH, the  $\alpha$ -MSH cells become more sensitive to TRH but not to CRH (14). These findings establish  $\alpha$ -MSH as a stress hormone in addition to adrenocorticotrophic hormone (ACTH) in the tilapia hypothalamus-pituitary-interrenal axis.

In fishes, three forms of  $\alpha$ -MSH are commonly found: des-, mono-, and diacetylated  $\alpha$ -MSH (10, 12). In tilapia, desacetylated  $\alpha$ -MSH is mainly a storage form, whereas mono- and diacetylated  $\alpha$ -MSH are secreted forms (12). The release of these two forms is regulated differentially: when the fish are exposed to low-pH water, the ratio of diacetyl  $\alpha$ -MSH/monoacetyl  $\alpha$ -MSH (di/mono ratio) in the blood plasma is elevated compared with control plasma. Because the diacetylated form has the highest intrinsic corticotropic activity (13), the di/mono ratio reflects the corticotropic quality of the  $\alpha$ -MSH signal. Of the stimulators tested, TRH, but not CRH, enhanced the di/mono ratio of the released  $\alpha$ -MSH. The present study focuses on the effects of exposing tilapia to water with a low pH on the dopaminergic control of the release of the amount of immunoreactive  $\alpha$ -MSH and the preferential release of diacetyl  $\alpha$ -MSH.

Dopamine is a potent inhibitor of  $\alpha$ -MSH release from the neurointermediate lobe (NIL) in mammals (see Ref. 26 for review), amphibians (27), and fish (20, 12). However, dopamine can also be a stimulatory secretagogue as was shown for goldfish growth hormone cells (6). Indeed, two distinct classes of receptor subtypes for dopamine, with opposite effects, are known: the first subtype consists of the dopamine D<sub>1</sub>-like receptors D<sub>1a</sub> and D<sub>1b</sub> and the recently described D<sub>1c</sub> receptor in lower vertebrates (15, 24). These are stimulatory receptors, coupled to adenylyl cyclase (AC), whereas the dopamine D<sub>2</sub>-like subtypes (D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub>) are inhibitory receptors coupled to AC or other second messenger systems (see Refs. 7 and 11 for review). Specific drugs are available to distinguish between the stimulatory and inhibitory receptor subtypes: SKF-38393 and 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (6-chloro-APB) specifically stimulate the D<sub>1</sub>-like dopamine receptors, whereas 2-hydroxy apomorphin (2-HA) and quinpirole act as specific D<sub>2</sub> dopamine receptor agonists.

Exposure of fish to water with low pH induced changes in the regulatory effects of dopamine on the MSH cells: low concentrations of dopamine exerted stimulatory effects and high concentrations exerted inhibitory effects on the release of  $\alpha$ -MSH, but only in

low pH-exposed fish (in control fish dopamine was only inhibitory at any concentration tested). This indicated the presence of two receptor subtypes on the MSH cells of low-pH tilapia. We here report on the effect of low-pH water on the  $D_1$ - and  $D_2$ -receptor activities in tilapia NILs. Furthermore, we assessed the effect of dopamine on the di/mono ratio of the released  $\alpha$ -MSH (i.e., the quality of the corticotropic signal) and studied the participation of the two dopamine receptor subtypes in this effect.

## MATERIALS AND METHODS

**Experimental animals.** Mature male tilapia, *Oreochromis mossambicus*, were obtained from laboratory stock. The fish were kept in 120-liter tanks with city of Nijmegen tap water at 26°C and pH 7.8, on a grey background. The photoperiod was 12 h of direct illumination alternating with 12 h of darkness. The body weights ranged from 100 to 250 g. The fish were fed a dried fish food (Trouvit, Trouw, Putten, the Netherlands). Feeding was stopped 24 h before an experiment.

**Exposure of fish to low-pH water.** Fish were kept in 120-liter tanks of tap water, and the pH was lowered from 7.8 to 4.5 at a constant rate over a 24-h period by addition of  $H_2SO_4$  (10 mM). The pH was controlled using pH-stat equipment (Radiometer PHM 83 + TTT 80 + ABU 80, Copenhagen, Denmark). They were kept at pH 4.5 for 7 days before experimentation. Control fish were kept in tap water of pH 7.8. Immediately after capture, the fish were killed by spinal transection. The animals exposed to low-pH water are referred to as low-pH tilapia.

**In vitro superfusions.** Freshly dissected pituitary glands were bisected into NIL and pars distalis (PD), with the use of a fine scalpel blade and a binocular microscope. Tissues were placed on a cheesecloth filter in a superfusion chamber and superfused with a *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered (15 mM; pH 7.38) Ringer solution containing 132 mM NaCl, 2 mM KCl, 2 mM  $CaCl_2 \cdot 2H_2O$  (2 mM), 0.25% (wt/vol) glucose, and 0.03% (wt/vol) bovine serum albumin. This medium was kept at 26°C and pumped through the chambers at a rate of 30  $\mu$ l/min by a Watson-Marlow 503U multichannel peristaltic pump (Smith and Nephew Watson-Marlow, Falmouth, Cornwall, UK); 10-min fractions were collected throughout the experiments. In line with previous findings, basal release of  $\alpha$ -MSH became consistently constant after 3 h (12), and from this time on dopamine or one of its analogs were added. In concentration-effect studies, medium supplemented with dopamine (Sigma, St. Louis, MO,  $10^{-14}$ – $10^{-5}$  M), SKF-38393, 6-chloro APB (RBI, Natick, MA,  $10^{-14}$ – $10^{-5}$  M), 2-HA, and quinpirole (RBI,  $10^{-9}$ – $10^{-6}$  M), was administered to the NILs for 30 min, the time span within which the peak response is always observed (12). To avoid interactions between the drugs, separate tissues were used for every concentration and drug. The fractions were collected with an Isco fraction collector. Fractions were frozen immediately after collection and stored at  $-20^\circ C$  until further assay. The release of  $\alpha$ -MSH was expressed in percentage of basal release, defined as the mean of the release seen from 150–180 min after the superfusion had been started.

**Determination of di/mono ratios.** Isolated NILs were preincubated in 1 ml HEPES-buffered saline for 2 h at 26°C. Next, the saline was replaced with 1 ml fresh saline, and the incubation was allowed to proceed for 1 h (the  $\alpha$ -MSH released during this first hour was used to calculate basal

release). Then the saline was replaced once more by 1 ml saline containing dopamine or one of the drugs, and after 1 h the saline was collected and the  $\alpha$ -MSH released determined. Samples were subjected to high-performance liquid chromatography (HPLC) analysis, and subsequently peak areas of the different forms of  $\alpha$ -MSH were determined by  $\alpha$ -MSH radioimmunoassay (RIA) (see below). Di/mono ratios were calculated by dividing the peak area of diacetyl  $\alpha$ -MSH by the peak area of monoacetyl  $\alpha$ -MSH. The di/mono ratio for controls found in this way was similar to previously published values obtained from static incubations (14) or a superfusion setup (12).

**Separation methods.** Samples from in vitro incubations were submitted to the HPLC application of the SMART system (Pharmacia, Uppsala, Sweden). Separation was performed on a reversed-phase column ( $\mu$ RPC  $C_{18}$ SC 2.1/10, Pharmacia). Products were eluted with a gradient of acetonitrile containing trifluoroacetic acid (TFA, 0.1% vol/vol) in  $H_2O$  containing TFA (0.1% vol/vol). The flow rate was 150  $\mu$ l/min. The three forms of  $\alpha$ -MSH eluted between 21 and 25% acetonitrile. Fractions (75  $\mu$ l) were collected, dried in a Savant Speedvac concentrator, and resolved in HCl (0.01 N)/methanol (1:1 vol/vol). The  $\alpha$ -MSH content of the fractions was determined by RIA.

**RIA for  $\alpha$ -MSH.** Concentrations of  $\alpha$ -MSH were determined in duplicate by RIA. The antiserum was raised against synthetic monoacetyl  $\alpha$ -MSH (Sigma, M4135) and characterized in our laboratory (13). Immunocytochemical experiments showed no cross-reaction of the antiserum with ACTH cells in the PD of tilapia. The cross-reactivity with desacetyl, monoacetyl, and diacetyl  $\alpha$ -MSH was 100%, with ACTH-(1–24) and ACTH-(1–39) <0.5%. The antiserum was used in a final dilution of 1:60,000. The  $\alpha$ -MSH was labeled with  $^{125}I$  (Amersham International, Amersham, Bucks, UK) using the iodogen method (21) and purified through solid-phase extraction (octadecyl Bakerbond column; J. T. Baker, Phillipsburg, NJ). Free labeled MSH was separated from immunocomplexed MSH by polyethylene glycol precipitation. The detection limit of the assay was 6 fmol  $\alpha$ -MSH. The interassay variation in these experiments was  $11 \pm 3\%$ ; the intra-assay variation was  $5 \pm 2\%$  ( $n = 8$ ).

**Data analysis and statistics.** Results are presented as means  $\pm$  SE. The effect of a secretagogue on  $\alpha$ -MSH release was calculated by determining the peak value of stimulation or inhibition in the time curve of the superfusion experiments as described in detail before (12). In the case of dopamine and the dopamine  $D_1$  agonists, the first five data points ( $10^{-15}$ – $10^{-11}$  M) were taken to calculate the concentrations giving 50% of the maximum responses ( $EC_{50}$ s). The data of the concentration-response studies were best fitted to the equation

$$R = [(R_0 + R_{\max}) * 10^{C-EC_{50}}] / (10^{C-EC_{50}} + 1)$$

(where  $R$  is the response of the MSH tissue,  $C$  is the concentration of secretagogue,  $R_0$  is the basal, unstimulated release rate, and  $R_{\max}$  is the maximum level of stimulation). A nonlinear data analysis program describing a sigmoid curve (16) was used. This software package allows the determination of three parameters from a minimum of four data points (degrees of freedom = 1; see also Ref. 14).

With respect to the other data, statistical significance of differences was assessed using the Student's  $t$ -test or the Mann-Whitney  $U$  test where appropriate.  $P < 0.05$  was taken as the fiducial limit.

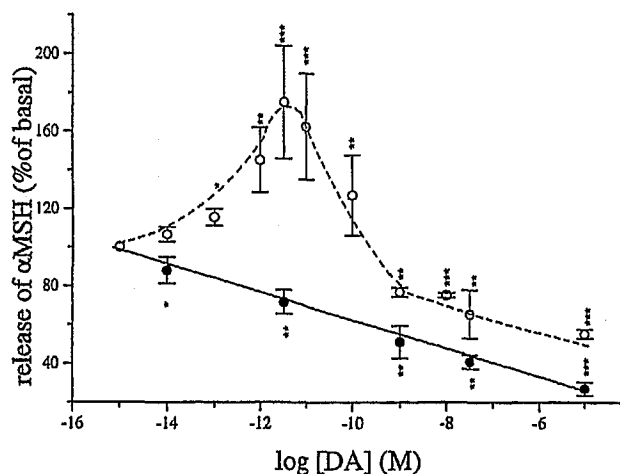


Fig. 1. Concentration-effect curves of dopamine (DA) showing maximal release of  $\alpha$ -melanophore-stimulating hormone (MSH) from control (●) and low-pH tilapia neurointermediate lobes (NILs) (○). Values are means  $\pm$  SE ( $n = 4-7$  preparations). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , significantly different from control (=100%) values.

## RESULTS

Figure 1 presents the concentration-effect curve of dopamine on NILs of control and low-pH tilapia. Basal release was  $127 \pm 21$  fmol/min per NIL ( $n = 44$ ) for controls and  $368 \pm 61$  fmol/min per NIL ( $n = 23$ ) in low-pH tilapias, and these values were designated 100%. When the concentration of dopamine was increased from  $10^{-13}$  up to  $10^{-5}$  M, a progressive inhibition was observed in control fish. An  $EC_{50}$  of  $10^{-8}$  M was calculated. In the low-pH tilapia, dopamine concentrations ranging from  $10^{-7}$  to  $10^{-5}$  M exerted an inhibitory effect on the release of  $\alpha$ -MSH in a concentration-dependent way. The  $EC_{50}$  derived from this part of the curve was  $10^{-6.9}$  M. In concentrations ranging from  $10^{-14}$  to  $10^{-11}$  M, dopamine stimulated the release of

$\alpha$ -MSH. The highest stimulation (175%) was observed at  $10^{-12}$  M dopamine. The  $EC_{50}$  for this stimulating effect was  $10^{-12.4}$  M. No stimulatory effect of dopamine was observed in controls.

Figure 2 shows the concentration-effect curves of the  $D_1$  agonists SKF-38393 (Fig. 2A) and 6-chloro APB (B) on control and low-pH tilapia. Both agonists were without effect in control fish. However, in low-pH tilapia a concentration-dependent stimulatory effect was evident in the range of  $10^{-14}$  to  $10^{-12}$  M, with a highest stimulation of 165% for both SKF-38393 and 6-chloro-APB, and  $EC_{50}$ s of  $10^{-12.6}$  and  $10^{-12.7}$  M, respectively. At concentrations greater than  $10^{-10}$  M and up to  $10^{-6}$  M, the stimulation of the  $\alpha$ -MSH release declined to  $\sim 140\%$  for both agonists.

In Figure 3, the effect of the  $D_2$  agonists quinpirole (Fig. 3A) and 2-HA (Fig. 3B) is shown on the release of  $\alpha$ -MSH from NILs of control and of low-pH adapted fish. Quinpirole inhibited the secretion of  $\alpha$ -MSH from control and low-pH NILs with equal efficiency. The  $EC_{50}$ s of quinpirole was  $10^{-8.1}$  and  $10^{-7.9}$  M for control and low-pH tissue, respectively. Also 2-HA had the same inhibiting effect on the release of  $\alpha$ -MSH for control and low-pH NILs. The  $EC_{50}$ s were  $10^{-9.2}$  and  $10^{-9.1}$  M in control and low-pH NILs, respectively.

Table 1 shows the di/mono ratios of  $\alpha$ -MSH recovered from the medium during a 1-h period of basal release and during a 1-h period in which dopamine ( $10^{-6}$  M), specific  $D_1$  ( $10^{-11}$  M), or  $D_2$  ( $10^{-6}$  M) agonists had been added, both for control and low-pH tilapia. The di/mono ratio of the unstimulated  $\alpha$ -MSH release from low-pH tilapia is significantly higher ( $P < 0.05$ ) than that released by controls. Only dopamine and the specific  $D_1$ -like agonists enhanced the di/mono ratios in  $\alpha$ -MSH released from low-pH NILs ( $P < 0.05$  and  $P < 0.01$ , respectively), but not from control NILs.

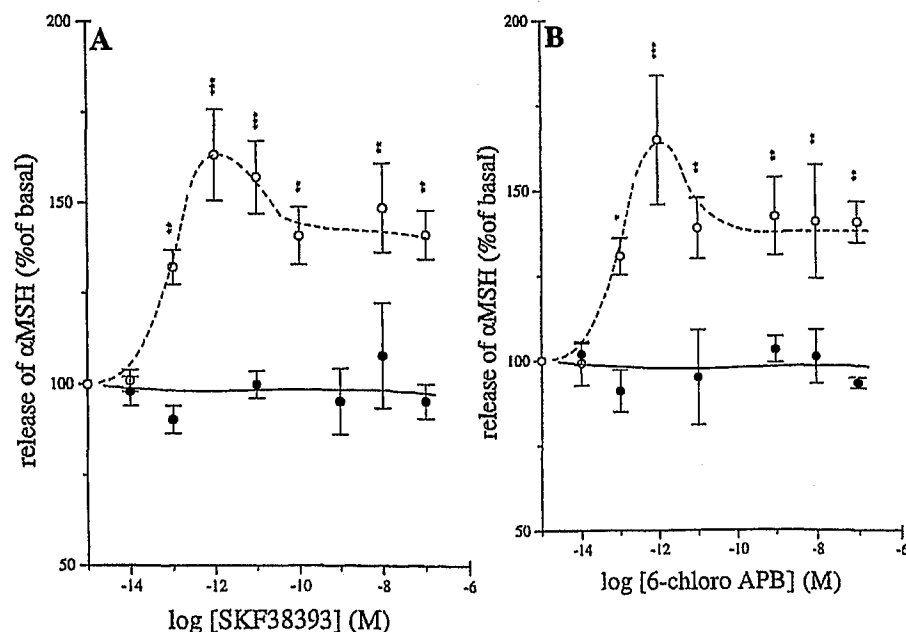
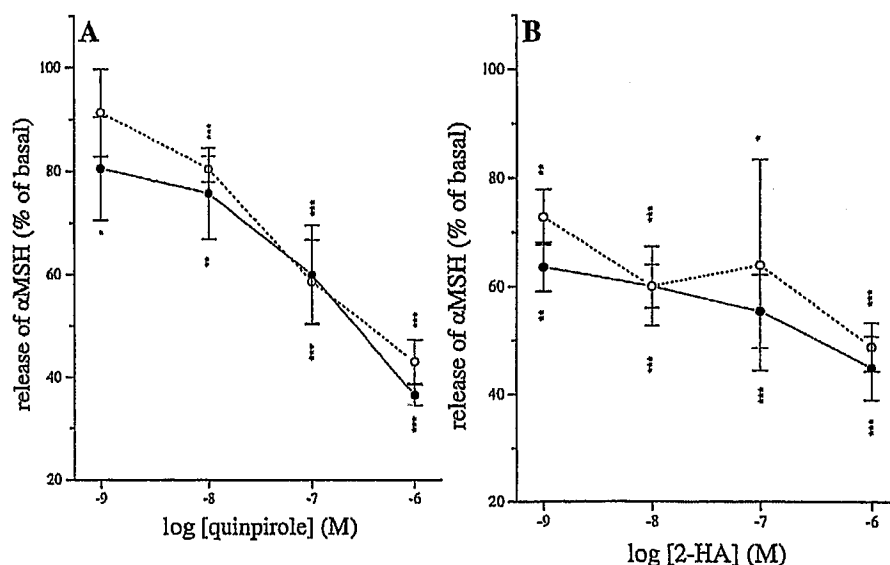


Fig. 2. Concentration-effect curves of  $D_1$  agonists SKF-38393 (A) and 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (6-chloro APB; B) showing maximal stimulation of  $\alpha$ -MSH release from control (●) and low-pH tilapia NILs (○). Values are means  $\pm$  SE ( $n = 5-8$  preparations). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , significantly different from control values.

Fig. 3. Concentration-effect curves of  $D_2$  agonists quinpirole (A) and 2-hydroxy apomorphin (2-HA; B) showing maximal inhibition of  $\alpha$ -MSH release from control (●) and low-pH tilapia NILs (○). Values are means  $\pm$  SE ( $n = 4-8$  preparations). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , significantly different from control values.



## DISCUSSION

The main conclusions drawn from this study are as follows. 1) In control fish, dopamine inhibits the release of  $\alpha$ -MSH. However, in low-pH tilapia, dopamine exerts both inhibitory and stimulatory effects on  $\alpha$ -MSH release. High concentrations ( $10^{-10}$ - $10^{-5}$  M) of dopamine inhibit and low concentrations ( $10^{-14}$ - $10^{-11}$  M) stimulate the release of  $\alpha$ -MSH. 2) Pharmacological studies indicate that the inhibitory effect of dopamine is exerted through a  $D_2$ -like receptor, which is not affected by low-pH conditions. 3) The stimulatory effect of dopamine is mediated through a  $D_1$ -like receptor. This  $D_1$ -like receptor could pharmacologically only be demonstrated in NILs of low-pH tilapia. 4) Activation of the  $D_1$ -like receptor by dopamine or its  $D_1$  receptor agonists increases the quality of the MSH corticotrophic signal.

**Two differential dopamine effects with distinct  $EC_{50}$ s.** Dopamine inhibited the release of  $\alpha$ -MSH from the NIL of tilapia in concentrations between  $10^{-10}$  and  $10^{-6}$  M. However, when tilapia had been exposed for 1 wk to low-pH water, dopamine showed two distinct effects. In the concentration range between  $10^{-10}$  and  $10^{-6}$  M,

dopamine inhibited the release of  $\alpha$ -MSH. However, the inhibition was smaller than in controls. In the lower concentration range ( $10^{-13}$ - $10^{-10}$  M), dopamine stimulated the release of  $\alpha$ -MSH. In vertebrates, two dopamine receptor subtypes with opposite functions are known: the stimulatory  $D_1$ -like and the inhibitory  $D_2$ -like receptors (7, 23). The stimulatory effect of dopamine on the release of  $\alpha$ -MSH can be explained by the activation of a  $D_1$ -like receptor. Apparently, this  $D_1$ -like receptor had been induced in NILs of low-pH tilapia. It has a higher affinity for dopamine than the inhibitory receptor.

**Effects of  $D_1$  agonists.** Two selective  $D_1$  agonists, SKF-38393 and 6-chloro-APB, had no effect between  $10^{-14}$  and  $10^{-6}$  M concentrations on the release of  $\alpha$ -MSH from NILs from control fish. Both agonists, however, stimulated the release of  $\alpha$ -MSH from the NILs of low-pH tilapia, in a concentration-dependent manner in the lower concentration range ( $10^{-14}$  M- $10^{-12}$  M). A peak response was observed around  $10^{-12.4}$  M, followed by a plateau, where the stimulation was 85% of the peak value. This biphasic effect could be explained by interactions of the agonists with other catecholamine receptor subtypes present in the NIL: synergistic as well as antagonistic interactions have been shown between  $D_1$  and  $D_2$  dopamine receptors (28). Another attractive explanation is that part of the  $D_1$ -like receptors change from a high-affinity state to a low-affinity state with the increase of the agonist concentration, as described in pituitary and brain tissue of mammals (for review, see Ref. 22). This change to the low-affinity state of the receptor would lead to a decreased occupancy of the receptors by the agonist and, hence, to a decrease in maximal stimulation of  $\alpha$ -MSH release.

Similar stimulatory effects of extremely low dopamine concentrations and inhibitory effects of high concentrations of dopamine have been reported for the release of prolactin from rat pituitary gland (4, 8, 19). This stimulatory effect of dopamine, however, could not

Table 1. Peak area di/mono ratios in culture medium (1 h static incubations) of control and low-pH tilapia, effects of dopamine ( $10^{-5}$  M), and selective  $D_1$  ( $10^{-11}$  M) and  $D_2$  ( $1.0 \mu$ M) agonists

	Controls	Low-pH Stressed
Basal	0.55 $\pm$ 0.03 (8)	0.75 $\pm$ 0.02* (8)
Dopamine	0.56 $\pm$ 0.02 (5)	0.93 $\pm$ 0.07† (6)
2-Hydroxy apomorphin	0.58 $\pm$ 0.03 (4)	0.73 $\pm$ 0.10 (5)
Quinpirole	0.52 $\pm$ 0.05 (6)	0.73 $\pm$ 0.05 (6)
SKF-38393	0.60 $\pm$ 0.03 (5)	0.95 $\pm$ 0.05† (9)
6-Chloro APB	0.59 $\pm$ 0.01 (6)	0.93 $\pm$ 0.06† (7)

Values are means  $\pm$  SE; nos. of experiments are shown in parentheses. di/mono ratios, diacetyl  $\alpha$ -melanophore-stimulating hormone (MSH)/monoacetyl  $\alpha$ -MSH ratios; 6-Chloro APB, 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide. \* $P < 0.05$  compared with control value; † $P < 0.05$  compared with low pH-stressed basal release (Mann-Whitney  $U$  test).

be mimicked by  $D_1$  agonists or by  $D_2$  agonists, but was blocked by the  $D_2$  antagonist eticlopride, indicating that the stimulatory effect is mediated indeed through a  $D_2$  rather than a  $D_1$  receptor (5). Stimulatory  $D_1$ -like receptor activity has recently been demonstrated in pituitary glands of another fish. Pharmacological (6) and receptor binding studies (29) corroborate the presence of a  $D_1$ -like receptor on goldfish growth hormone cells in the pituitary distal lobe. Interestingly and in accordance with our findings, the receptor binding studies by Wong and co-workers (29) showed no binding of the  $D_1$ -antagonist SCH-23390 in the NIL of goldfish kept at neutral (control) pH, comparable to our control fish. Apparently, pharmacological agents that may be successfully used in studies on mammals cannot always be used in lower vertebrates, and this indicates that significant evolution of dopamine receptors has occurred.

**Effects of  $D_2$ -agonists.** The specific  $D_2$  agonists quinpirole and 2-HA mimicked the inhibitory effect of dopamine on the release of  $\alpha$ -MSH in control fish. This indicated the presence of a  $D_2$ -like receptor on the MSH cells. There is consensus that the inhibitory effect of dopamine is mediated through  $D_2$ -like receptors in amphibians (27) and mammals (2). Quinpirole and 2-HA had comparable and inhibitory effects on the release of  $\alpha$ -MSH from NILs of control and low-pH tilapia. This shows that a  $D_2$ -like receptor is responsible for the inhibitory effect of dopamine on the release of  $\alpha$ -MSH; this receptor is equally sensitive to either of these agonists. Dopamine activates both  $D_1$  and  $D_2$  receptors and, indeed, the dopamine concentration-effect curve for low-pH tilapia (Fig. 1) can be considered a composition of the separate curves of the  $D_1$  agonists (Fig. 2) and the  $D_2$  agonists (Fig. 3). The observation that NILs of low-pH tilapia require higher concentrations of dopamine for inhibition of MSH release (compared with controls) must, therefore, be attributed solely to the induction of the  $D_1$ -like receptor.

**Enhancement of corticotrophic quality of  $\alpha$ -MSH by  $D_1$ -like agonists.** We have demonstrated earlier that the corticotrophic quality of  $\alpha$ -MSH is positively correlated with an increase of the di/mono ratio of its molecular forms (13). The di/mono ratio of  $\alpha$ -MSH released in vitro in low-pH fish is higher than in control animals. It was shown that TRH elevates the di/mono ratio in controls. In low-pH tilapia TRH can still exert its di/mono ratio elevating effect (14). In control fish, dopamine does not have any effect on the di/mono ratio. Interestingly, in low-pH tilapia dopamine does elevate the di/mono ratio. These effects can be mimicked by the specific  $D_1$  agonists SKF-38393 and 6-chloro APB but not by the  $D_2$  agonists 2-HA and quinpirole. We consider this as a further indication that the di/mono ratio elevation is a property of  $D_1$ -like receptor activity.

### Perspectives

The blood plasma level of  $\alpha$ -MSH was enhanced during acid stress, and the action of  $\alpha$ -MSH may contribute to a stimulated cortisol release in these fish (13). Therefore, the induction of the  $D_1$ -like dopamine receptor during low-pH adaptation may well be of

physiological significance, because the secretion of an inhibitor of  $\alpha$ -MSH release may be expected to decrease during stress. It is generally found that the different dopamine systems in the brain are selectively modulated by different kinds of stressors (1, 9). Indeed, the activity of the rat tuberohypophyseal dopaminergic neurons that project to the NIL (3) decreases during restraint, leading to a reduced dopamine concentration in the intermediate lobe. The diminished dopamine concentration in the vicinity of the MSH cells is accompanied by an increased  $\alpha$ -MSH secretion (17, 18). Therefore, we suggest that dopamine concentrations that we have shown to be stimulatory in vitro occur in the vicinity of the MSH cells in the NIL of acid stressed tilapia. This means that in low pH tilapia, dopamine in fact stimulates the release of  $\alpha$ -MSH and enhances the corticotrophic quality of  $\alpha$ -MSH. Dopamine may exclusively operate as an inhibitor of the  $\alpha$ -MSH release under control conditions.

Our data do not allow a definite identification of the here-described  $D_1$ -like receptor as a  $D_{1a}$ , a  $D_{1b}$ , or a  $D_{1c}$  receptor, because these  $D_1$ -like receptor subtypes cannot be distinguished pharmacologically. However, because of the very high affinity of the receptor, we speculate that it is a  $D_{1b}$  receptor rather than a  $D_{1a}$  receptor, because the  $D_{1b}$  receptor has a much higher affinity for dopamine than the  $D_{1a}$  receptor (11, 25). The lack of pharmacological characterization of the recently described  $D_{1c}$  receptor (15) in tilapia prevents us from speculating as to the identification of the here-described  $D_1$ -like receptor as one of the  $D_{1c}$  subtype. Further identification on molecular biological level is presently in progress.

The mechanism that controls the induction of the  $D_1$ -like receptor activity is not clear.

An attractive hypothesis to test is that the increased plasma levels of TRH in the low-pH tilapia (14) not only influence the pituitary MSH cells but also enhance the activity of the hypothalamus-hypophysis-thyroid axis and that the increased activity of this axis underlies the induction of the stimulatory dopamine receptor on the MSH cell. Expression studies will eventually answer the question of whether control occurs at transcriptional or posttranscriptional level. This is the first study to show the presence of  $D_1$ -like receptor activity in the pituitary NIL. The adaptation of tilapia to low-pH stress provides an elegant model to study the regulation of the dopamine  $D_1$ -like receptor activity.

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Received 19 July 1996; accepted in final form 4 February 1997.

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