5-Hydroxytryptamine-induced calcium-channel gating in rainbow trout (*Oncorhynchus mykiss*) peripheral blood lymphocytes

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The present study was conducted on peripheral blood lymphocytes of rainbow trout (*Oncorhynchus mykiss*) to assess the role of 5-hydroxytryptamine (5-HT; 'serotonin') in calcium signalling. 5-HT-induced increases in intracellular free calcium concentrations, $[Ca^{2+}]_i$, and its action was mediated by 5-HT receptor subtype 3 (5-HT₃), but not by 5-HT receptor subtype 1A (5-HT_{1A}) or subtype 2 (5-HT₂) in these cells. In Ca^{2+} -containing medium (1 mM $CaCl_2$), 5-HT and 2-methyl-5-HT (5-HT₃ receptor agonist) induced increases in $[Ca^{2+}]_i$, whereas in Ca^{2+} -free medium (0 Ca^{2+} , 1 mM EGTA), these two agents failed to evoke

increases in $[Ca^{2+}]_i$ in these cells, demonstrating that 5-HT mobilizes Ca^{2+} from the extracellular environment. Furthermore, 5-HT-induced increases in $[Ca^{2+}]_i$ are not contributed to by the intracellular endoplasmic reticulum (ER) pool, as thapsigargin, an agent that recruits Ca^{2+} from ER stores, had additive effects on 5-HT-induced $[Ca^{2+}]_i$ responses in fish peripheral lymphocytes. 5-HT-induced increases in $[Ca^{2+}]_i$ were mediated by 5-HT₃ receptors via gating the calcium through L-type, but not N-type, calcium channels in trout lymphocytes.

INTRODUCTION

5-Hydroxytryptamine (5-HT; 'serotonin') is a neurotransmitter present in the brain, regulating a great number of physiological mechanisms such as sleep, appetite, thermoregulation, control of pituitary secretions and behaviour [1]. In peripheral organs, 5-HT is present in high concentrations in platelets, basophils, mast cells and gastrointestinal mucous [2]. 5-HT can also affect immune functions and several findings suggest that modulation of the immune system by 5-HT occurs at the level of lymphocytes [3–5]. 5-HT administration to mice in which the serotonergic neurons were destroyed, diminished the production of anti-(sheep red blood cell) antibodies, suggesting that there might be a direct effect of this biogenic amine *in vivo* on immunocompetent cells [6]. Similarly, addition of exogenous 5-HT *in vitro* was found to suppress phytohaemagglutinin-induced blastogenesis [7]. 5-HT has also been shown to augment interferon- γ -induced phagocytosis [8] and natural killer cell cytotoxicity [9]. Besides, pharmacological data have suggested a multiplicity of 5-HT receptors at the lymphocyte level [3–5], and molecular biology data have confirmed unequivocally the existence of multiple serotonergic receptors [10].

Furthermore, hyperactivity in brain 5-HT metabolism and increased levels of its metabolite, 5-hydroxyindole acetic acid, in the urine have been reported in subjects exposed to different stressful conditions [11–13]. Since stress has been shown to alter immune functions [14–16], a role for 5-HT in this immunomodulation has been proposed [2].

As in mammals, beside immunocytochemical localization [17,18], the presence of low- and high-affinity serotonergic receptors in the brain of rainbow trout has been demonstrated [19], and different types of stresses such as predator exposure [20],

hierarchical dominance, agonistic behaviour or starvation [21–23] have been reported to induce an increase in brain serotonergic activity in these animals. In analogy to mammals, it is possible that free 5-HT in the blood may influence the functioning of immunocompetent cells due to the fact that, in contrast with mammals, 5-HT can cross the blood–brain barrier in teleost fish [24]. We have recently shown that 5-HT exerts immunosuppressive effects on lipopolysaccharide- and phytohaemagglutinin-stimulated proliferation of rainbow trout peripheral blood lymphocytes via functional serotonergic receptors of subtype 1A (5-HT_{1A}) [25]. As regulation of intracellular free calcium concentration ($[Ca]_i$) has been proposed to be an important mechanism implicated in the transmission of cell surface activation signals coupled with diverse intracellular processes that culminate in lymphocyte proliferation in mammals [26–28], and as Evans et al. [29] have shown that the activity of the non-specific cytotoxic lymphocytes of channel catfish (*Ictalurus punctatus*) is modulated by $[Ca^{2+}]_i$, the present study was undertaken to investigate the effects of 5-HT on calcium signalling in fish peripheral blood lymphocytes (PBLs).

MATERIALS AND METHODS

Chemicals

Culture medium RPMI 1640 (Dutch modification) was obtained from Gibco, France. 5-HT, *R*-(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin [*R*-(+)-8-OH-DPAT], 2-methyl-5-HT, metoclopramide, ketanserin tartrate and sulpiride were purchased from Research Biochemicals. BSA, EGTA, ionomycin, thapsigargin, verapamil and diltiazem, were from Sigma. ω -Conotoxin and indo-1 acetoxymethyl ester (Indo-1/AM) were

Abbreviations used: 5-HT, 5-hydroxytryptamine ('serotonin'); 5-HT₃, 5-hydroxytryptamine receptor of subtype 3; 5-HT_{1A}, 5-hydroxytryptamine receptor of subtype 1A; 5-HT₂, 5-hydroxytryptamine receptor of subtype 2; PBL, peripheral blood lymphocyte; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)-tetralin; $[Ca^{2+}]_i$, intracellular free calcium concentration; ER, endoplasmic reticulum.

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Figure 1 Effects of 5-HT, its agonists and its antagonists on calcium signalling in fish PBLs

PBLs (2×10^6 cells/ml) were loaded with Indo-1/AM as described in the Materials and methods section. The effect of 5-HT (**A**), 8-OH-DPAT (**B**) and 2-methyl-5-HT (2-M-5-HT) (**C**), at the concentrations indicated below the arrows, on $[Ca^{2+}]_i$ were assessed. In the rest of the traces, the agents were used at concentrations as follows: metoclopramide (MCP), 20 μ M; 2-M-5-HT, 20 μ M; ketanserin (Ket), 10 μ M; sulpiride (Sulp) 50 μ M; 5-HT, 50 μ M.

obtained from Molecular Probes. Ficoll-Paque was from Pharmacia.

Experimental animals

Rainbow trout (*Oncorhynchus mykiss*) ranging in weight from 150 to 250 g and in length from 18 to 22 cm were obtained from a local hatchery (M/S Skiba, Haute-Vienne, France) and maintained in 1000-litre indoor circular glass-fibre tanks with a constant renewal of water (12 ± 2 °C) for 4 weeks before experimentation. They were fed five times weekly *ad libitum* with commercial food (Trouvit HD).

$[Ca^{2+}]$ measurements

To avoid stress, the fish were anaesthetized by placing them into a water tank containing 2-phenoxyethanol (0.3 ml/l) and bled from the caudal vein using a heparinized syringe. Blood was then quickly diluted with 5 vol. of culture medium (RPMI 1640 Dutch modification) and layered on to an equal volume of Ficoll-Paque. After 40 min of centrifugation at 1000 *g* and 4 °C, the interface containing PBLs was collected and washed twice (800 *g*, 10 min) in culture medium.

The PBL cell density was adjusted to 2×10^6 cells/ml in the incubation buffer which contained the following, as described by

Evans et al. [29]: NaCl, 140.3 mM; KCl, 5 mM; CaCl₂, 1 mM; MgCl₂, 1 mM; Hepes, 20 mM; glucose, 10 mM and Na₂HPO₄, 1 mM, pH 7.4. Cells were loaded with Indo-1/AM (2 μ M) in identical buffer, containing 2% BSA, for 45 min at 20 °C. After incubation, the cells were washed twice (800 g, 10 min) in 20 ml of buffer and remained suspended in identical buffer without BSA. For experiments in Ca²⁺-free medium (0 Ca²⁺), CaCl₂ was replaced by 1 mM EGTA.

[Ca²⁺]_i was measured according to Grynkiewicz et al. [30] in a Kontron spectrofluorimeter (SFM 25) with monochromator settings of 345 nm (excitation) and 405 nm (emission). Addition of the test molecules to the cuvette was made with no interruptions in recordings. [Ca²⁺]_i was calculated by using the equation:

$$[\text{Ca}^{2+}]_i = K_d(F - F_{\min}) / (F_{\max} - F)$$

A value of 250 nM for the apparent K_d of Ca²⁺ binding to Indo-1 was applied. The test molecules were added to the cuvette at the times indicated by arrows in the Figures. For studies on calcium-channel blockers, the cells were preincubated for 15 min before [Ca²⁺]_i measurements.

In the Figures, single traces are shown but similar results have been obtained in at least five separate experiments. Means $\pm$ S.D. of increases in [Ca²⁺]_i are reported in Figure 6, and data were analysed with Student's *t*-test of significance.

RESULTS

Addition of 5-HT (25 or 50 μ M) to suspensions of fish PBLs loaded with Indo-1/AM caused an increase in fluorescence intensity that can be attributed to a rise in [Ca²⁺]_i [30]. The magnitude of the 5-HT response depended on the concentration

of this monoamine: at 50 μ M, 5-HT caused an increase in [Ca²⁺]_i of 120 ± 17.6 nM (Figures 1A and 6A). To assess the specific action of 5-HT, we employed different agonists and antagonists of serotonergic receptors. Figure 1(B) shows that 8-OH-DPAT, a 5-HT_{1A} receptor specific agonist, at 25 or 50 μ M failed to induce increases in [Ca²⁺]_i. Interestingly, 2-methyl-5-HT (20 μ M), a 5-HT₃ receptor agonist, evoked a rise in [Ca²⁺]_i of 72 ± 21 nM (Figures 1C and 6A). Prior addition of the 5-HT₃ receptor antagonist metoclopramide at 20 μ M to these cells abolished significantly the increase in [Ca²⁺]_i induced by 2-methyl-5-HT (Figures 1D and 6A). Two antagonists of the 5-HT receptor of subtype 2 (5-HT₂), ketanserin at 10 μ M and sulpiride at 50 μ M, failed to block significantly the increase in [Ca²⁺]_i evoked by 5-HT (Figures 1E, 1F and 6A).

Thapsigargin, an inhibitor of Ca²⁺-ATPase of the endoplasmic reticulum (ER), at 1 μ M increased [Ca²⁺]_i to 190 ± 6.4 nM in fish lymphocytes (Figures 2A and 6B). The thapsigargin-induced response in [Ca²⁺]_i was more prolonged than that elicited by 5-HT, the maximum level being attained only after 1 or 2 min. 5-HT and thapsigargin had additive effects on the increases in [Ca²⁺]_i (Figures 2B and 2C), and the 5-HT-induced response in [Ca²⁺]_i was not significantly modified by prior addition of thapsigargin (Figure 6B).

Figures 3(A) and 3(B) show that in Ca²⁺-free buffer (0 Ca²⁺), 5-HT and 2-methyl-5-HT failed to induce increases in [Ca²⁺]_i in fish lymphocytes. The amplitudes of increases in [Ca²⁺]_i were significantly reduced for each compound in Ca²⁺-free buffer (Figure 6C). Thapsigargin-induced elevation in [Ca²⁺]_i was higher in Ca²⁺-containing medium (1 mM CaCl₂) than that in Ca²⁺-free medium (0 Ca²⁺) (Figure 3D). Furthermore, the response to 5-HT was not influenced by pretreatment with thapsigargin in Ca²⁺-free medium (0 Ca²⁺) (Figure 3C).

Figure 2 Effects of 5-HT and thapsigargin on [Ca²⁺]_i in fish PBLs

PBLs (2×10^6 cells/ml) were loaded with Indo-1/AM as described in the Materials and methods section. The test molecules, thapsigargin (Thap), 1 μ M, and 5-HT, 50 μ M, were added at the times indicated by arrows.

Figure 3 Effects of 5-HT, 2-methyl-5-HT (2-M-5-HT) and thapsigargin on $[Ca^{2+}]_i$ in fish PBLs in Ca^{2+} -containing (1 mM $CaCl_2$) and Ca^{2+} -free (0 Ca^{2+} but 1 mM EGTA) medium

PBLs (2×10^6 cells/ml) were loaded with Indo-1/AM as described in the Materials and methods section. The test molecules, thapsigargin (Thap), 1 μ M, 5-HT, 50 μ M, were added at the times indicated by arrows.

In the following experiments, by employing the Ca^{2+} -channel blockers we explored the mechanisms by which 5-HT brings about rises in $[Ca^{2+}]_i$. Hence, lanthanum (La^{3+}) and nickel (Ni^{2+}), agents known to block voltage-dependent Ca^{2+} channels [31], curtailed significantly 5-HT-induced increases in $[Ca^{2+}]_i$ when used at 100 μ M (Figures 4A, 4B and 6D). Furthermore, verapamil and diltiazem (inhibitors of L-type calcium channels) completely abolished the effects of 5-HT (Figures 4C, 4D and 6D) and 2-methyl-5-HT (Figures 4F, 4G and 6D) on $[Ca^{2+}]_i$ in these cells. On the other hand, ω -conotoxin (an inhibitor of N-type calcium channels) failed to influence significantly the effect of 5-HT (Figures 4E and 6D) and 2-methyl-5-HT on $[Ca^{2+}]_i$ (results not shown). The specific Ca^{2+} -channel blockers were not used at more than 1 μ M because, at high concentrations, these compounds lose their specificity [32].

In Figures 5(A) and 5(B), we used the Ca^{2+} -ionophore, ionomycin, to elucidate further the role of intra/extracellular Ca^{2+} stores in generating the Ca^{2+} signal upon 5-HT application. At 100 nM, ionomycin caused a fast rise in $[Ca^{2+}]_i$ of

800 ± 121.4 nM (Figure 6E). After the addition of the ionophore, the magnitude of Ca^{2+} signals caused by 5-HT (Figure 5A) and 2-methyl-5-HT (Figure 5B) was not significantly curtailed (Figure 6E). After 100 nM ionomycin, thapsigargin (1 μ M) failed to increase significantly the $[Ca^{2+}]_i$ in these cells (Figures 5C and 6E), indicating the depletion of internal stores by ionomycin treatment. Furthermore, 5-HT (50 μ M) failed to evoke a rise in $[Ca^{2+}]_i$ after ionomycin treatment in Ca^{2+} -free buffer (Figures 5D and 6E).

DISCUSSION

In this study, 5-HT increased $[Ca^{2+}]_i$ in Indo-1-loaded fish PBLs. 8-OH-DPAT, an agonist of the 5-HT_{1A} receptor subtype, failed to induce an increase in $[Ca^{2+}]_i$, indicating that 5-HT_{1A} is not implicated in the increases in $[Ca^{2+}]_i$ in these resting lymphocytes. These observations are substantiated by our recent findings in

Figure 4 Effects of calcium-channel blockers on $[Ca^{2+}]_i$ in fish PBLs

PBLs (2×10^6 cells/ml) were loaded with Indo-1/AM as described in the Materials and methods section. Before Ca^{2+} measurements, cells were incubated for 15 min in the presence of one of the following: La^{3+} , 100 μM (A); Ni^{2+} , 100 μM (B); verapamil, 1 μM (C and G); diltiazem, 1 μM (D and F) or ω -conotoxin, 1 μM (E). The test molecules, 5-HT, 50 μM , or 2-methyl-5-HT (2-M-5-HT), 20 μM , were added at the times indicated by arrows.

which we have demonstrated that only mitogen-stimulated, but not resting, trout peripheral lymphocytes express 5-HT_{1A} receptors [25]. Similarly, sulpiride and ketanserin, antagonists of 5-HT₂ receptors, failed to diminish the increases in $[Ca^{2+}]_i$ induced by 5-HT. On the other hand, 2-methyl-5-HT, a specific agonist of the 5-HT₃ receptor subtype, induces an increase in $[Ca^{2+}]_i$, and this increase is antagonized by pretreatment with metoclopramide, a 5-HT₃ receptor antagonist. These observations demonstrate that increases in $[Ca^{2+}]_i$ in fish lymphocytes are brought about via activation of 5-HT₃ receptors. In support of these observations, we can cite the findings of Reiser et al. [33] who have demonstrated that 5-HT₃ receptor stimulation increases $[Ca^{2+}]_i$ by triggering the influx of extracellular calcium into a neuronal cell line (neuroblastoma $\times$ glioma hybrid cells).

In fact, an increase in $[Ca^{2+}]_i$ may be achieved by one of three mechanisms. First, Ca^{2+} may be mobilized from intracellular stores such as mitochondria or ER [26]. Secondly, the rate of Ca^{2+} extrusion from the cytoplasm may be slowed, allowing an increase in $[Ca^{2+}]_i$ as Ca^{2+} leaks into the cell down its steep electrochemical gradient [34,35]. Finally, plasma membrane

calcium channels may open, allowing a rapid influx of this ion [36]. These processes are not mutually exclusive and may work in concert for a particular response in a given cell type.

To establish the route of $[Ca^{2+}]_i$ activation by 5-HT, experiments using the Ca^{2+}_i ionophore, ionomycin, were carried out [37]. Pollock et al. [38] have shown that re-sequestration of internally released Ca^{2+} did not occur in platelets treated with 1 μM ionomycin. Thus in the continuous presence of this ionophore, the internal stores are short-circuited and recovery of an elevated $[Ca^{2+}]_i$ is presumably almost entirely due to extracellular Ca^{2+} intrusion [38]. We observed that addition of ionomycin (100 nM) to fish lymphocytes in Ca^{2+} -containing medium evoked increases in $[Ca^{2+}]_i$, and prior addition of this antibiotic had no effect on the responses to 5-HT and 2-methyl-5-HT, suggesting that 5-HT-induced increases in $[Ca^{2+}]_i$ are contributed to by Ca^{2+} from the extracellular medium. This finding can be further supported by the following observations: in the Ca^{2+} -free medium, 5-HT failed to induce increases in $[Ca^{2+}]_i$ in lymphocytes incubated with ionomycin. Whether or not calcium channels are involved in the increases in $[Ca^{2+}]_i$ via

Figure 5 Effects of different agents after ionomycin treatment on $[Ca^{2+}]_i$ in fish PBLs

PBLs (2×10^6 cells/ml) were loaded with Indo-1/AM as described in the Materials and methods section. The test molecules, ionomycin (Iono), 100 nM, 5-HT, 50 μ M, 2-methyl-5-HT (2-M-5-HT), 20 μ M, and thapsigargin (Thap), 1 μ M, were added at the times indicated by arrows. Insets to (A) and (B) and main Figure (C) show the effects of 5-HT, 2-M-5-HT and thapsigargin after addition of Iono. Experiments in (D) were performed in Ca^{2+} -free buffer.

serotonergic receptor stimulation was investigated by using the inhibitors of voltage-sensitive calcium channels. Hence Ni^{2+} and La^{3+} , known to block these calcium channels [31], abolished the increases in $[Ca^{2+}]_i$ induced by 5-HT, implying a role of calcium channels in the influx of Ca^{2+} . Furthermore, by employing specific calcium-channel blockers like verapamil and diltiazem (L-type calcium-channel blockers) and ω -conotoxin (N-type calcium-channel blocker), we observed that L-type, but not N-type, calcium channels are implicated in the 5-HT- and 2-methyl-5-HT-induced increases in $[Ca^{2+}]_i$ in fish lymphocytes. The observation that the response to 5-HT depends on the opening of transmembrane calcium channels is further supported by the lack of 5-HT effects in the absence of extracellular Ca^{2+} . Neither 5-HT nor 2-methyl-5-HT induced a rise in $[Ca^{2+}]_i$ in Ca^{2+} -free medium. These findings corroborate the electrophysiological studies of Bolanos et al. [39], who have shown that the 5-HT₃ receptor subtype incorporates a ligand-gated ion channel similar to the nicotinic receptor ion channel complex. The presence of calcium channels in fish PBLs is not surprising, as the heterogeneity of calcium-channel subtypes is a more widespread phenomenon than previously thought and is not limited to neurons [9,40].

Thapsigargin, a sesquiterpene lactone tetraester derived from *Thapsia gargonica*, has been shown to increase $[Ca^{2+}]_i$ by a

mechanism different from that involved in myo-inositol 1,4,5-trisphosphate-induced calcium release by inhibiting ER Ca^{2+} -ATPase [41]. In the present study, thapsigargin induced a rise in $[Ca^{2+}]_i$ in fish lymphocytes. Addition of thapsigargin and 5-HT exerted additive effects on the increases in $[Ca^{2+}]_i$ in fish lymphocytes. These results demonstrate that, in fish lymphocytes, Ca^{2+} may be mobilized from intracellular stores such as the ER, but 5-HT does not act on the thapsigargin-sensitive pool. Furthermore, the thapsigargin-evoked elevation in $[Ca^{2+}]_i$ was higher and more sustained in cells in a Ca^{2+} -containing medium than in those in a Ca^{2+} -free medium. Hence the second phase, i.e. sustained Ca^{2+} elevation, induced by thapsigargin seems to be contributed by Ca^{2+} influx in accordance with the capacitative model of Ca^{2+} entry; hence an increase in $[Ca^{2+}]_i$ leads directly to Ca^{2+} influx in order to compensate the intracellular pool [41].

We believe that this is the first report that characterizes the functional mobilization of Ca^{2+} stores in fish lymphocytes. As 5-HT is a very important monoamine in rainbow trout, implicated in gonadodrophic maturation [42] and osmoregulation in gill and gut via 5-HT₃ [43], we can infer that this neurotransmitter may modulate trout immune functions during seasons or stressful conditions by acting on specific 5-HT receptors, particularly 5-HT₃ receptor subtype, coupled with Ca^{2+} gating, and hence altering the Ca^{2+} homeostasis in fish leucocytes.

Figure 6 Increases in $[Ca^{2+}]_i$ induced by different agents in fish PBLs

The test molecules were added at the following concentrations: serotonergic agonists (5-HT, 50 μ M and 2-M-5-HT, 20 μ M), and antagonists, ketanserin (Ket), 10 μ M, sulpiride (Sulp), 50 μ M and metoclopramide (MCP), 20 μ M; thapsigargin (Thap), 1 μ M, ionomycin (Iono), 100 nM and different Ca^{2+} -channel blockers, La³⁺, 100 μ M; Ni²⁺, 100 μ M; verapamil (Verap), 1 μ M; diltiazem (Dilt), 1 μ M and ω -conotoxin (ω -C), 1 μ M. In (C), 'yes' denotes the presence of Ca^{2+} (1 mM $CaCl_2$), and 'not' denotes the absence of Ca^{2+} (0 Ca^{2+} but 1 mM EGTA) in the medium. Values are means $\pm$ S.D. of five experiments in each assay. Data were analysed by employing Student's *t*-test of significance. Values differ significantly compared with their respective control: $P < 0.005$ (**) and $P < 0.0001$ (***).

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REFERENCES

- 1 McGeer, P. J., Eccles, J. C. and McGeer, E. G. (1987) in *Molecular Neurobiology of the Mammalian Brain*, 2nd edn., pp. 319–346, Plenum Press, New York
- 2 Jankovic, B. D. (1989) *Immunol. Lett.* **21**, 101–118
- 3 Aune, T. M., Kelly, K. A., Ranges, G. E. and Bombara, M. P. (1990) *J. Immunol.* **145**, 1826–1831
- 4 Aune, T. M., Grath, M. C., Sarr, K. M., Bombara, M. P. and Kelley, K. A. (1993) *J. Immunol.* **151**, 1175–1183.
- 5 Khan, N. A., Ferriere, F. and Deschaux, P. (1995) *Cell. Immunol.* **165**, 148–152
- 6 Jackson, J. C., Cross, R. J., Walker, R. F., Markesbery, W. R., Brooks, W. J. and Roszman, T. L. (1985) *Immunology*, **54**, 505–512
- 7 Khan, I. A., Bhardwaj, G., Watal, M. C. and Agarwal, S. C. (1986) *Int. Arch. Allergy Immunol.* **81**, 378–380
- 8 Sternberg, E. M., Trial, J. and Parker, C. W. (1986) *J. Immunol.* **137**, 276–282
- 9 Hellstrand, K. and Hermodsson, S. (1987) *J. Immunol.* **139**, 869–875
- 10 Peroutka, S. J. (1993) *J. Neurochem.* **60**, 408–416
- 11 Khan, N. A. and Kalra, V. (1988) *Immunol. Ther. Exp.* **36**, 717–721
- 12 Pucilowski, O. and Kostowski, W. (1983) *Behav. Brain Res.* **9**, 33–48
- 13 Sarkar, F. H., Singh, R. H. and Udupa, K. N. (1977) *Experientia* **33**, 417–418
- 14 Besedovsky, H. O., Rey, A. E. D. and Sorkin, E. (1985) *J. Immunol.* **135**, 750S–754S
- 15 Fleshner, M., Watkins, L. R., Lockwood, L. L., Bellgrau, D., Laudenslager, M. L. and Maier, S. F. (1992) *J. Neuroimmunol.* **41**, 131–142
- 16 Harbuz, M. S. and Lightman, S. L. (1992) *J. Endocrinol.* **134**, 327–339
- 17 Bolliet, V. and Ali, M. A. (1992) *Brain Behav. Evol.* **40**, 234–240
- 18 Holmqvist, B. I. and Ekström, P. (1995) *J. Chem. Neuroanat.* **8**, 125–145
- 19 Khan, N. A., Troutaud, D. and Deschaux, P. (1996) *Neurosci. Res. Commun.* **18**, 97–105
- 20 Winberg, S., Myrberg, A. A. and Nilsson, G. E. (1993) *NeuroReport* **4**, 399–402
- 21 Winberg, S. and Nilsson, G. E. (1993) *Comp. Biochem. Physiol.* **106C**, 597–614
- 22 Winberg, S., Nilsson, G. E. and Olsen, K. H. (1992) *J. Exp. Biol.* **165**, 229–239
- 23 Winberg, S., Nilsson, G. E. and Olsen, K. H. (1992) *J. Comp. Physiol.* **170**, 93–99
- 24 Fritsche, R., Reid, S. G., Thomas, S. and Perry, S. F. (1993) *J. Exp. Biol.* **178**, 191–204
- 25 Ferriere, F., Khan, N. A., Troutaud, D. and Deschaux, P. (1996) *Dev. Comp. Immunol.* **20**, 273–283
- 26 Hesketh, T. R., Moore, J. P., Morris, J. D. H., Taylor, M. V., Rogers, J., Smith, G. A. and Metcalfe, J. C. (1985) *Nature (London)* **313**, 481–484
- 27 Lichtman, A. H., Segel, G. B. and Lightman, M. A. (1983) *Blood* **61**, 413–418
- 28 Rasmussen, H., Kujima, I., Kujima, K., Zawalich, W. and Appeldorf, W. (1984) *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **18**, 159–162
- 29 Evans, D. L., Harris, D. T. and Jaso-Friedmann, L. (1992) *Dev. Comp. Immunol.* **16**, 383–394
- 30 Gryniewicz, G., Poenie, M. and Tsien, R. Y. (1985) *J. Biol. Chem.* **260**, 3440–3450
- 31 Tsien, R. W. (1983) *Annu. Rev. Physiol.* **45**, 341–358
- 32 Codignola, A., Tarroni, P., Clementi, F., Pollo, A., Lovallo, M., Carbone, E. and Sher, E. (1993) *J. Biol. Chem.* **268**, 26240–26247
- 33 Reiser, G., Donié, F. and Binmöller, F. J. (1989) *J. Cell Sci.* **93**, 545–555
- 34 Tolloczko, B., Jia, Y. L. and Martin, J. G. (1995) *Am. J. Physiol.* **269**, L234–L240
- 35 Prpic, V., Green, K. C., Blackmore, P. F. and Exton, J. H. (1984) *J. Biol. Chem.* **259**, 1382–1387
- 36 Hagiwara, S. and Byerly, L. (1981) *Annu. Rev. Neurosci.* **4**, 69–73
- 37 Liu, C. and Hermann, T. E. (1978) *J. Biol. Chem.* **253**, 5892–5894
- 38 Pollock, W. K., Sage, S. O. and Rink, T. J. (1987) *FEBS Lett.* **210**, 132–136
- 39 Bolanos, F. J., Schechter, L. E., Miquel, M. C., Emerit, M. B., Rumigny, J. F., Hamon, M. and Gozlan, H. (1990) *Biochem. Pharmacol.* **40**, 1541–1550
- 40 Rampe, D. and Triggle, D. J. (1990) *Trends Pharmacol. Sci.* **11**, 112–115
- 41 Thastrup, O., Cullen, P. J., Drobak, B. K., Hanley, M. R. and Dawson, A. P. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2466–2470
- 42 Saligaud, G., Salbert, G., Bailhache, T., Bennani, S. and Jegou, P. (1992) *Gen. Comp. Endocrinol.* **85**, 261–268
- 43 Mori, Y. and Ando, M. (1991) *J. Comp. Physiol.* **161B**, 387–392

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