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Modulation by calcineurin of 5-HT_3 receptor function in NG108-15 neuroblastoma × glioma cells

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1 We have investigated the mechanism of regulation of 5-HT_3 receptor channel sensitivity in voltage-clamped (−80 mV) NG108-15 neuroblastoma cells.

2 The 5-HT-induced inward current activated rapidly. The fast onset was followed by a biphasic decay which was characterized by two time constants, τ_1 (1.1±0.21s) and τ_2 (8.9±1.6s), respectively. Brief applications of 5-HT, applied at 2 min intervals, induced a decrease in the amplitude of the 5-HT_3 receptor-mediated peak inward currents.

3 Buffering of intracellular calcium with the calcium chelator BAPTA (10 mM) instead of EGTA (10 mM) attenuated the 5-HT-induced loss of responsiveness of 5-HT_3 receptors. Omission of calcium from the extracellular medium yielded a similar attenuation of loss of responsiveness.

4 Inclusion of the protein kinase inhibitor, staurosporine (1 μM) or of okadaic acid (1 μM), an inhibitor of protein phosphatases 1 and 2A, in the intracellular buffer solution did not affect 5-HT_3 receptor sensitivity.

5 Injection of cyclosporin A-cyclophilin A complex (20 nM), which potently inhibits calcineurin, did not affect the time constants of the biphasic decay of the 5-HT response τ_1 (1.4±0.28s) and τ_2 (11.3±1.7s). The complex, however, prevented the loss of 5-HT_3 receptor responsiveness upon repeated application of 5-HT. A similar, but weaker effect was observed after intracellular application of the autoinhibitory peptide domain of calcineurin (1 μM).

6 The recovery of desensitized 5-HT_3 receptors upon a second application of 5-HT (1 μM) showed a half-life time ($\tau_{\frac{1}{2}}$) of 2.6±0.12 min in control cells which was reduced to 1.6±0.09 min in cells treated with cyclosporin A-cyclophilin A (20 nM) complex.

7 We conclude that calcineurin does not affect the fast decay of the 5-HT_3 receptor response but may be involved in a slower process which regulates channel activity.

Keywords: 5-HT_3 receptors; calcineurin; NG108-15 cells; receptor desensitization

Introduction

Most ligand-gated ion channels have been shown to be regulated by protein phosphorylation. For example, phosphorylation of γ- and δ-nicotinic receptor subunits by protein kinase C (PKC), induces receptor desensitization (Huganir & Greengard, 1983). Similarly phosphorylation of β and γ_2 subunits of the $GABA_A$ receptor mediates desensitization of GABA-induced chloride currents (Kirkness *et al.*, 1989). In cerebellar Purkinje cells, AMPA receptor responsiveness has been negatively correlated to PKC-mediated phosphorylation (Linden *et al.*, 1991). In contrast, the responsiveness of other ligand-gated channels such as NMDA and kainate receptors is enhanced after phosphorylation of serine residues by PKC, calmodulin-dependent kinase II or protein kinase A (MacDonald *et al.*, 1989).

Recent findings indicate a role of phosphatases in the control of ligand-gated receptor activity. It has been reported that the sensitivity of NMDA receptor channels is regulated by a calcium-dependent phosphatase, presumably calcineurin (Lieberman & Mody, 1994). Also $GABA_A$ (Chen *et al.*, 1990) and voltage-dependent calcium channels (Chad & Eckert, 1986) receptors may be modulated by calcineurin.

A possible role of calcium in the regulation of 5-HT_3 receptor sensitivity has been suggested (Peters *et al.*, 1988; Robertson & Bevan, 1991; Yakel *et al.*, 1993). The 5-HT_3 receptor channel has structural and functional similarity to ligand-gated channels such as the kainate and the nicotinic acetylcholine receptor (Maricq *et al.*, 1991). Like these channels, the sensitivity of 5-HT_3 receptors has been shown to be modulated by adenosine 3′:5′-cyclic monophosphate (cyclic AMP), most likely via protein kinase A (Yakel & Jackson, 1988). The effect of protein phosphatases on 5-HT_3 receptor sensitivity has received little attention until now. Therefore, in this study we have investigated the possible influence of protein-serine kinases and phosphatases, in particular the calcium/calmodulin dependent phosphatase calcineurin upon 5-HT_3 receptor function. A preliminary account of this work has already appeared in abstract form (Arbuckle *et al.*, 1993).

Methods

NG108-15 cells were cultured as described previously (Docherty *et al.*, 1991). Briefly, cells were grown in 3.5 mm culture dishes in DMEM supplemented with 10% foetal calf serum and 0.5% penicillin-streptomycin solution. Three days prior to the experiment the cells were differentiated in DMEM supplemented with 1% foetal calf serum, 0.5% penicillin-streptomycin solution, 10 μM prostaglandin E_1 and 50 μM 1-isobutyl 3-methylxanthine (IBMX).

The cells were studied at room temperature (20–22°C) in a chamber perfused at a rate of 2–3 ml min^{-1}. Whole cell recording techniques were used to record membrane currents using an EPC-7 amplifier (List Electronics). Pipette resistances were between 3 and 5 MΩ. Cells were voltage clamped at −80 mV and recordings were performed on approximately spherical cells with few or no processes and a diameter of 30–

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50 μm. The intracellular solution contained (in mM): NaCl 5, $CaCl_2$ 2, $MgCl_2$ 1.8, KCl 130, HEPES 40 and EGTA 10 (pCa approx 7.7; pH = 7.4). In some experiments EGTA was replaced by 10 mM BAPTA (pCa approx 7.9). The composition of the extracellular electrolyte solution was (in mM): NaCl 120, $CaCl_2$ 2.5, $MgCl_2$ 1, KCl 6, $NaHCO_3$ 22, HEPES 5, glucose 5; pH = 7.4. In some experiments calcium was omitted from the extracellular solution and EGTA (2 mM) was added. In order to minimize dialysis of the cell contents the application of 5-HT was started less than 1 min after breaking into the cell. 5-HT (10 μM) was applied for 5 s by either low pressure ejection (3–4 psi) from a micropipette placed 5–10 μm from the cell, or by a u-tube. The 5-HT-mediated inward currents were recorded and analyzed with pClamp software (Axon instruments). The decay constants of the responses were measured by the bi-exponential fit procedure provided in the pClamp software package; the 'goodness of fit' was evaluated by the 'least squares' method. All data are presented as means ± s.e.mean. Statistical significance ($P<0.05$) was evaluated by analysis of variance and Student's t tests.

Compounds used

BAPTA (1,2-*bis*(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid), 5-HT (5-hydroxytryptamine hydrochloride) and staurosporine were purchased from Sigma St. Louis M.O. U.S.A.; okadaic acid sodium salt was purchased from Anawa, Switzerland; cyclosporin A, cyclophilin A and autoinhibitory peptide; (H-Ile-Thr-Ser-Phe-Glu-Glu-Ala-Lys-Pro-Met-Arg-Glu-Asn-Ile-Arg-Asp-Asp-Leu-Gly-Pro-Arg-Arg-Asp-Ala-Met-Pro-OH; 97% purity) were manufactured at Sandoz Chemical laboratories. Stock solutions (10 mM) of okadaic acid, staurosporine and cyclosporin A were prepared in a 10% (vol/vol) dimethylsulphoxide (DMSO) solution. In all experiments the final DMSO concentrations did not exceed 0.2%.

Results

The response to 5-HT

Application of 5-HT (10 μM, 5 s) to differentiated NG108-15 cells evoked an inward current with an average peak amplitude of 1.52 ± 0.19 nA ($n=45$). Typically, the 5-HT-induced inward current had a rapid (< 300 ms) onset followed by a relatively slow decay. The decay of the current, measured during a 60 s application of 5-HT was biphasic with the time constants $\tau_1 = 1.1 \pm 0.21$s ($n=8$) and $\tau_2 = 8.9 \pm 1.6$s ($n=8$). When 5-HT was applied as a series of three or four brief (5s) applications, given at 2 min intervals (see Figure 1), then the amplitude of the second response was significantly ($P<0.05$) reduced with respect to the first response. The response to the first and second responses were 1.52 ± 0.19 and 0.38 ± 0.13 nA, respectively ($n=45$), i.e. there was a 75% decrease in amplitude of the second response. Surprisingly, there was no significant further reduction in the current amplitude ($P>0.05$) when 5-HT was applied for a third or fourth time in a series. Mean amplitudes were 0.31 ± 0.12 for the third ($n=45$) and 0.32 ± 0.11 ($n=45$) for the fourth ($n=45$) response to 5-HT.

Compared to the first response, a small, insignificant decrease of the decay constant τ_1 of the second, third and fourth response to 5-HT was observed (see Table 1). There were also no significant changes in the decay constant τ_2.

In order to evaluate possible run down due to loss of high energy phosphates in one set of experiments, ATP (2 mM) was included in the patch pipette solution. Addition of ATP did not affect the decrease of the second, third and fourth response to 5-HT (Figure 1). Qualitatively similar data were obtained when 10 mM BAPTA was included instead of EGTA which was used for control experiments (see Methods). The amplitude of the first response to 5-HT was not significantly different from control cells but there was a significantly smaller

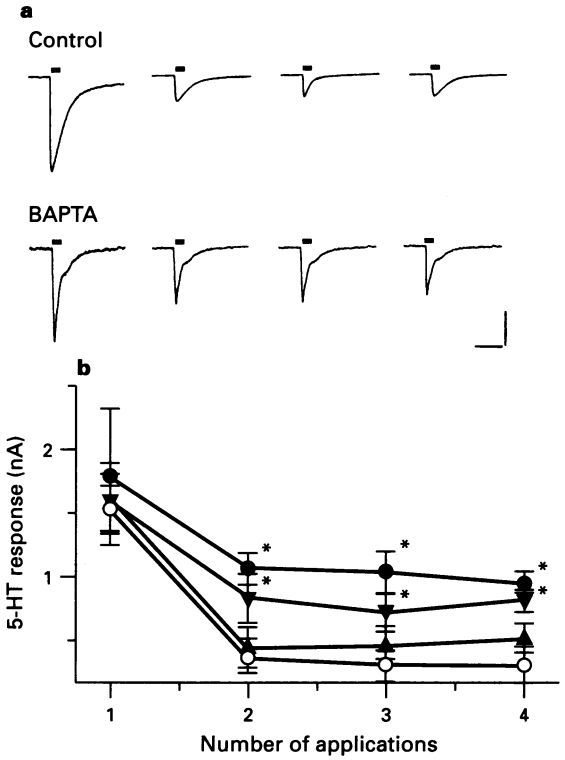

Figure 1 (a) Example of 4 repeated applications of 5-HT (10 μM, 5 s) given at 2 min intervals to an NG108-15 cell, under control conditions (i.e. with 10 mM EGTA in the pipette solution) or with BAPTA (10 mM) in the pipette solution. The horizontal bars indicate the application of 5-HT. The vertical and horizontal calibration bars represent 0.5 nA and 5 s, respectively. (b) Curves of the repeated application of 5-HT, under control conditions (○), with BAPTA 10 mM (●), with ATP (▲) in the patch-pipette solution and in calcium-free extracellular medium (▼). The data represent mean values ± s.e.mean ($n=17-45$). *Significant ($P<0.05$) differences between BAPTA-treated cells or cells recorded in calcium-free medium and control cells.

Table 1 Summary of the decay time constants (s), τ_1 and τ_2, of currents induced by application of 5-HT (5s) under control conditions and after treatment with BAPTA or CsA-Cp complex

Application	*1*	*2*	*3*	*4*
Control τ_1	1.3 ± 0.28 (n = 8)	1.0 ± 0.26 (n = 8)	0.8 ± 0.17 (n = 8)	0.8 ± 0.11 (n = 8)
Control τ_2	10.4 ± 1.1 (n = 8)	10.1 ± 1.4 (n = 8)	8.3 ± 1.6 (n = 8)	9.7 ± 1.4 (n = 8)
BAPTA τ_1	1.2 ± 0.21 (n = 5)	1.0 ± 0.17 (n = 4)	0.7 ± 0.19 (n = 4)	0.9 ± 0.18 (n = 4)
BAPTA τ_2	11.9 ± 1.90 (n = 5)	12.3 ± 1.8 (n = 4)	11.4 ± 1.5 (n = 4)	11.6 ± 1.9 (n = 4)
Complex τ_1	1.4 ± 0.28 (n = 9)	1.1 ± 0.22 (n = 9)	0.9 ± 0.14 (n = 7)	1.0 ± 0.17 (n = 7)
Complex τ_2	11.3 ± 1.7 (n = 9)	10.4 ± 1.1 (n = 9)	10.9 ± 1.4 (n = 7)	11.1 ± 1.3 (n = 7)

decrease in the amplitude of the second and subsequent responses to 5-HT in the series ($P<0.05$, see Figure 1) i.e. replacement of EGTA in the recording pipette solution with BAPTA helped to preserve 5-HT responsiveness. For the second, third and fourth response to 5-HT a small but not significant decrease of the decay constant τ_1 was observed (Table 1). Omission of calcium in the extracellular solution also partially conserved 5-HT responsiveness, albeit to a lesser extent than observed with BAPTA (Figure 1).

In order to study the recovery of loss of responsiveness of 5-HT$_3$ receptors, the effect of a second application of 5-HT at increasing time intervals was studied. Figure 2 illustrates the recovery in amplitude of the second response as a function of the time-application interval. After application of 5-HT (10 μM, 5 s), the response to a second application of 5-HT (10 μM, 5 s) was clearly reduced. The recovery from this loss of responsiveness was time-dependent (Figure 2) and the $t_{\frac{1}{2}}$ of the recovery was 2.6 ± 0.12 min ($n=8$).

Inhibition of calcineurin

The responsiveness to 5-HT was studied when cells were dialyzed with a complex of cyclosporin A (20 nM) and cyclophilin A (20 nM) which were added to the solution in the recording pipette. This complex has been shown to be a potent and highly selective inhibitor of the calcium- and calmodulin-dependent enzyme, calcineurin (Liu *et al.*, 1991). The peak current amplitude in response to the first application of 5-HT was significantly ($P<0.05$) increased by $102\pm14\%$ compared to control cells. Also there was only $35\pm15\%$ reduction in the peak amplitude of the second response to 5-HT compared to the first response (see Figure 3) i.e. there was significantly less reduction ($P<0.05$) than occurred in control experiments (see above). However the decay time constants τ_1 and τ_2 of the response to 5-HT were not significantly different from control (see Table 1). In cells dialyzed with cyclosporin A (20 nM) and cyclophilin A (20 nM) the rate of recovery of responsiveness after a first exposure to 5-HT was significantly accelerated such that $\tau_{\frac{1}{2}}=1.6\pm0.09$ min ($P<0.05$, $n=9$, see Figure 2).

Responses to 5-HT in cells dialyzed with either cyclosporin A alone ($n=14$, Figure 3) or cyclophilin A alone (data not shown) were not significantly different from responses observed in control cells. Intracellular application of the autoinhibitory peptide domain of calcineurin (1 μM) had an effect qualitatively similar but quantitatively smaller than the cyclosporin A–cyclophilin A complex (see Figure 3). The peak amplitude of the first 5-HT response was significantly increased ($P<0.05$, $n=11$) and there was a reduced loss of responsiveness when 5-HT was applied again after 2 min.

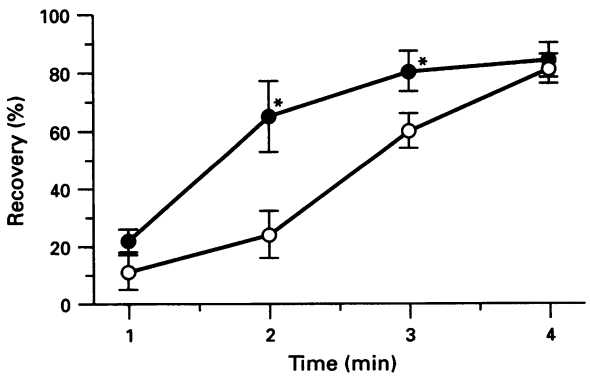

Figure 2 Time-dependent recovery of desensitized 5-HT$_3$ receptors upon a second application of 5-HT (5 s, 10 μM), in control cells (○) and in cells treated with cyclosporin A-cyclophilin A complex (●; 20 nM). The data represent mean values ± s.e.mean ($n=8-12$). *Significant ($P<0.05$) differences between cyclosporin A-cyclophilin A complex treated and control cells.

Other inhibitors of protein kinases and phosphatases

Inclusion of the non-specific protein kinase inhibitor, staurosporine (1 μM) in the intracellular solution did not prevent the loss of 5-HT$_3$ receptor sensitivity upon repeated application of 5-HT. During a series of three responses to 5-HT (10 μM, 5 s) applied at 2 min intervals to cells which were pretreated with extracellular applied staurosporine (1 μM, 30 min) the decline of the response was as great or greater than in untreated cells (Figure 4). These data suggest that the loss of 5-HT responsiveness is not due to phosphorylation by a serine/threonine protein kinase. Also intracellular application (1 μM) of the selective inhibitor of protein phosphatase 1 and 2A, okadaic acid (Bialojan & Takai, 1988), did not significantly ($P>0.05$) affect the decline in 5-HT$_3$ receptor sensitivity seen with repeated 5-HT application (Figure 4).

Discussion

Administration of 5-HT to NG108-15 cells induces an inward current with a fast onset followed by a biphasic decay. We assume that under the conditions used in this study the decay of the 5-HT response results from the fast desensitization of 5-HT$_3$ receptor channels. The τ_1 and τ_2 values found in this study are somewhat larger than those previously reported by Yakel *et al.* (1993) which may be the result of slightly different experimental procedures. After repeated short (5 s) applications of 5-HT, given at 2 min intervals, a strong decline of the response to 5-HT was observed, which reflects a prolonged desensitization of 5-HT$_3$ receptors. In the rest of this study we have investigated the mechanism of this steady-state desensi-

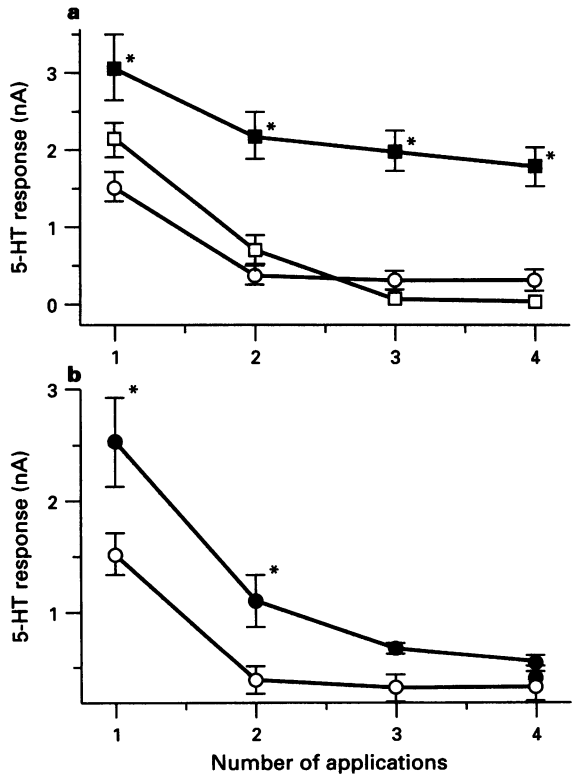

Figure 3 (a) Curves of the repeated application of 5-HT (5 s, 10 μM), under control conditions (○), with cyclosporin A (20 nM; □) or with cyclosporin A-cyclophilin A complex (20 nM; ■) included in the intracellular solution, respectively. (b) Curves of the repeated application of 5-HT (5 s, 10 μM), under control conditions (○), or with autoinhibitory peptide (1 μM; ●) included in the intracellular solution. The data represent mean values ± s.e.mean ($n=11-45$). *Significant ($P<0.05$) differences between control cells and cells pretreated with either CsA-Cp complex or autoinhibitory peptide.

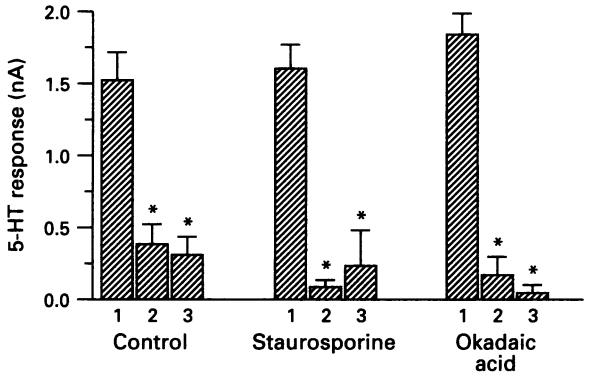

Figure 4 Graphs showing the mean amplitude of responses to application of 5-HT (5 s, 10 μM), repeated at 2 min intervals, under control conditions, in presence of staurosporine (1 μM) or okadaic acid (1 μM) which were included in the intracellular solution. The data represent mean values ± s.e.mean ($n = 11-13$). *Significant differences ($P < 0.05$) between the first and the second or third application within each group.

tization. One possible explanation for the decline of the responses is rundown caused by a loss of high energy phosphates. Addition of ATP to the patch pipette solution did not affect the 5-HT$_3$-receptor desensitization, suggesting that loss of ATP is not responsible for the decrease in current amplitude. We suggest that compartmentalized ATP levels may be sufficient to support local phosphorylation and dephosphorylation of the receptor. Substitution of EGTA with an equal concentration of BAPTA which chelates calcium more rapidly (Tsien, 1980) in the pipette solution partially preserved the response to repeated application of 5-HT in NG108-15 cells. This effect of BAPTA suggests that 5-HT$_3$ receptor sensitivity is modified by a calcium-dependent mechanism. Also omission of calcium from the extracellular solution partially conserved 5-HT$_3$ receptor responsivity which implies that under normal conditions calcium might enter via the 5-HT$_3$ channel. It has been shown that the 5-HT$_3$ channel is permeable to calcium (Yang, 1990). In addition, Peters *et al.* (1988) and Robertson & Bevan (1991), have reported that the amplitude of the maximal 5-HT-induced current amplitude is related to the concentration of extracellular calcium.

Since many effects on channel behaviour are mediated by protein phosphorylation, we have investigated the effects of protein kinase and phosphatase inhibitors on 5-HT$_3$-receptor-mediated channel desensitization. Intracellular application of the non-specific protein kinase inhibitor staurosporine did not affect the loss of sensitivity of the 5-HT$_3$ receptor upon multiple application of 5-HT suggesting that protein serine/threonine kinase activity is not critically involved in the desensitization. Our data are in contrast to those of Robertson & Bevan (1991), who found that staurosporine *per se* inhibits the 5-HT$_3$ receptor response. At present we have no explanation for this discrepancy, which may depend on the type of cell used. Intracellular application of okadaic acid, a potent and selective inhibitor of protein serine phosphatase 1 and 2A (Bialojan & Takai, 1988), did not affect receptor sensitivity and our data, therefore, suggest no involvement of dephosphorylation by phosphatase 1 or 2A. In order to investigate further a possible role of protein serine/threonine phosphatases we have investigated the effect of inhibitors of the calcium/calmodulin dependent protein phosphatase calcineurin. It has been reported that the immunosupressive drugs, cyclosporin A and FK-506, when associated with their respective binding proteins, cyclophilin A and FKBP, are potent and highly selective inhibitors of calcineurin (Liu *et al.*, 1991). Intracellular application of the complex of cyclosporin A and cyclophilin A to NG108-15 cells clearly preserved 5-HT$_3$ receptor function upon multiple application of 5-HT. Thus, a major finding presented here is that calcineurin reduces the sensitivity of the 5-HT$_3$ receptors.

As an alternative approach to study the involvement of calcineurin in 5-HT$_3$ receptor sensitivity, we investigated the effect of intracellular administration of the 25 amino-acid sequence of the autoinhibitory domain of calcineurin. This treatment preserved 5-HT-induced responses with a qualitatively similar, albeit less potent effect than the combination of cyclosporin A and cyclophilin A.

The peak inward current evoked by a first application of 5-HT in cells treated with the cylosporin A-cyclophilin A complex was significantly ($P < 0.05$) larger than in control cells. This result suggests that the 5-HT$_3$ receptor channels either spend more time in the open state or a resting level of channel inactivation due to a basal activity of calcineurin exists. Accordingly, after inhibition of calcineurin more channels would be available and activated by application of 5-HT. Both possibilities support the observation that calcineurin is involved in the control of cellular sensitivity to 5-HT.

The τ_1 and τ_2 values of the 5-HT responses were not significantly changed by pretreatment with either BAPTA or cyclosporin A-cyclophilin A complex (see Table 1). Thus inhibition of calcineurin does not appear to affect the fast desensitization of the receptor.

In an attempt to investigate further the effect of calcineurin on the desensitized state of 5-HT$_3$ receptors we have monitored the amount of time required for the 5-HT response to recover after one application of 5-HT. From Figure 2 it is clear that after inhibition of calcineurin, a faster recovery from desensitization is observed, suggesting that calcineurin enhances the steady-state desensitization of the 5-HT$_3$ response. Up to 5 min after the first application of 5-HT only a partial recovery (81 ± 9%) was observed.

It has been suggested that the activity of NMDA-receptor channels (Lieberman & Mody, 1994), capsaicin-activated channels (Yeats *et al.*, 1992) and of voltage-dependent calcium channels (Armstrong, 1989) may also be regulated by calcineurin. Accordingly, it is possible that modulation by calcineurin may apply to other ion channels, particularly those channels that are permeable to calcium.

The present results suggest that intracellular calcineurin activity may be an important factor determining the sensitivity of cells to 5-HT. This mechanism is not likely to be important for fast desensitization of 5-HT receptors, which still occurs even in the presence of inhibitors of calcineurin but presents a novel mechanism for the control of 5-HT$_3$ receptor function.

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References

ARBUCKLE, J.A., MEIGEL, I., BODDEKE, H.W.G.M. & DOCHERTY, R.J. (1993). Inhibition of calcineurin reduces desensitization of 5-HT$_3$ receptors in NG108-15 neuroblastoma × glioma hybrid cells. *J. Physiol.*, **473**, 267P.

ARMSTRONG, D.L. (1989). Calcium channel regulation by calcineurin, a Ca^{2+}-activated phosphatase in mammalian brain. *Trends Neurosci.*, **12**, 117–122.

BIALOJAN, C. & TAKAI, A. (1988). Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. *Biochem. J.*, **256**, 283–290.

CHAD, J.E. & ECKERT, R. (1986). An enzymatic mechanism for calcium current inactivation in *Helix* neurones. *J. Physiol.*, **378**, 31–52.

CHEN, Q.X., STELZER, A., KAY, A.R. & WONG, R.K.S. (1990). $GABA_A$ receptor function is regulated by phosphorylation in acutely dissociated guinea-pig hippocampal neurones. *J. Physiol.*, **420,** 207–22.

DOCHERTY, R.J., ROBBINS, J. & BROWN, D.A. (1991). NG108-15 neuroblastoma × glioma hybrid cell line as a model neuronal system. In *Cellular Neurobiology: a Practical Approach.* ed. Chad, J. & Wheal, H. Oxford: IRL Press. p. 75–120.

HUGANIR, R.L. & GREENGARD, P. (1983). cAMP-dependent protein kinase phosphorylates the nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **80,** 1130–1134.

KIRKNESS, E.F., BOVENKERK, C.F., UEDA, T. & TURNER, A.J. (1989). Phosphorylation of τ-aminobutyrate (GABA)/benzodiazepine receptors by cyclic AMP-dependent protein kinase. *Biochem. J.*, **259,** 613–616.

LIEBERMAN, D.N. & MODY, I. (1994). Regulation of NMDA channel function by endogenous CA^{2+}-dependent phosphatases. *Nature*, **369,** 235–239.

LINDEN, D.J., DICKINSON, M.H., SMEYNE, M. & CONNOR, J.A. (1991). A long-term depression of AMPA currents in cultured cerebellar purkinje neurons. *Neuron*, **7,** 81–89.

LIU, J., FARMER, J.D., LANE, W.S., FRIEDMAN, J., WEISSMAN, I. & SCHREIBER, S.L. (1991). Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell*, **66,** 807–815.

MACDONALD, J.F., MODY, I. & SALTER, M.W. (1989). Regulation of N-methyl-D-aspartate receptors revealed by intracellular dialysis of murine neurones in culture. *J. Physiol.*, **414,** 17–34.

MARICQ, A.V., PETERSON, A.S., BRAKE, A.J. & JULIUS D. (1991). Primary structure and functional expression of the 5-HT$_3$ receptor, a serotonin-gated ion channel. *Science*, **254,** 432–437.

PETERS, J.A., HALES, T.G. & LAMBERT, J.J. (1988). Divalent cations modulate 5-HT$_3$ receptor-induced currents in NE-115 neuroblastoma cells. *Eur. J. Pharmacol.*, **151,** 491–495.

ROBERTSON, B. & BEVAN, S. (1991). Properties of 5-hydroxytryptamine$_3$ receptor-gated currents in adult rat dorsal root ganglion neurones. *Br. J. Pharmacol.*, **102,** 272–276.

TSIEN, R.Y. (1980). New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis and properties of prototype structures. *Biochemistry*, **16,** 2396–2404.

YAKEL, J.L. & JACKSON, M.B. (1988). 5-HT$_3$ receptors mediate rapid responses in cultured hippocampus and a clonal cell line. *Neuron*, **I,** 615–621.

YAKEL, J.L., LAGRUTTA, A., ADELMAN, J.P. & NORTH, R.A. (1993). Single amino acid substitution affects desensitization of the 5-hydroxytryptamine type 3 receptor expressed in Xenopus oocytes. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 5030–5033.

YANG, J. (1990). Ion permeation through 5-hydroxytryptamine-gated channels in Neuroblastoma N18 cells. *J. Gen. Physiol.*, **96,** 1177–1198.

YEATS, J.C., BODDEKE, H.W.G.M. & DOCHERTY, R.J. (1992). Capsaicin desensitization in rat dorsal root ganglion neurones is due to activation of calcineurin. *Br. J. Pharmacol.*, **107,** 238P.

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