Full-length and truncated Kv1.3 K^+ channels are modulated by 5-HT_{1c} receptor activation and independently by PKC

JAYASHREE AIYAR, STEPHAN GRISSMER, AND K. GEORGE CHANDY
Department of Physiology and Biophysics, University of California, Irvine, California 92717

Aiyar, Jayashree, Stephan Grissmer, and K. George Chandy. Full-length and truncated Kv1.3 K^+ channels are modulated by 5-HT_{1c} receptor activation and independently by PKC. *Am. J. Physiol.* 265 (*Cell Physiol.* 34): C1571–C1578, 1993.—In T-cells, the Shaker-related gene, Kv1.3 encodes the type *n* K^+ channel, whereas the type *l* channel is a product of the Shaw subfamily gene, Kv3.1. Both these genes are also expressed in the brain. We have used the *Xenopus* oocyte heterologous expression system to study the modulatory effects of serotonin (5-hydroxytryptamine, 5-HT) on both these cloned channels. In oocytes coexpressing the mouse 5-HT_{1c} receptor and mouse Kv1.3 channel, addition of 100 nM 5-HT causes a complete and sustained suppression of Kv1.3 currents in ~20 min. In contrast, 5-HT has no effect on mouse Kv3.1 currents when coexpressed with 5-HT_{1c} receptor. The 5-HT-mediated suppression of Kv1.3 currents proceeds via activation of a pertussis toxin-sensitive G protein and a subsequent rise in intracellular Ca^{2+}, but Ca^{2+} does not directly block the channel. Protein kinase (PK) C activation is not part of the pathway linking 5-HT_{1c} receptor to Kv1.3 channels. However, phorbol esters independently suppress Kv1.3 currents. Deletion of the first 146 amino acids from the NH_2-terminal, containing putative tyrosine kinase and PKA phosphorylation sites, does not alter the time course of 5-HT-mediated suppression of Kv1.3 currents, indicating that these residues are not necessary for modulation. Treatment of oocytes with calmodulin or phosphatase inhibitors does not alter 5-HT-mediated modulation. Collectively, these experiments indicate that the mouse Kv1.3 channel is capable of being modulated by 5-HT via 5-HT_{1c} receptor in a G protein and Ca^{2+}-dependent manner, but the subsequent steps in the pathway remain elusive. PKC also suppresses Kv1.3 currents but by an independent pathway.

Shaker-related gene; Shaw-related gene; T-cells; G protein; calcium; *Xenopus* oocytes; protein kinase C; serotonin

NEUROTRANSMITTERS AND HORMONES have diverse effects on cells of the immune system (1). One such neurotransmitter, serotonin (5-hydroxytryptamine, 5-HT), suppresses mitogen-induced lymphocyte activation (7), augments delayed-type hypersensitivity (2), enhances natural killer cell cytotoxicity (17), induces an increase in cytosolic Ca^{2+} in T-lymphocytes (5), and downregulates macrophage Ia expression (29). The mechanisms mediating these processes are currently unclear. In the nervous system, 5-HT exerts its physiological effects by interacting with a variety of specific 5-HT receptors that are coupled to distinct G protein and second messenger pathways (19). The 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors couple to adenylate cyclase, whereas the 5-HT_{1c} and 5-HT_2 receptors activate phospholipase C and induce the release of intracellular Ca^{2+} (19). The 5-HT_3 receptor is a ligand-gated ion channel (22). In the brain, one consequence of 5-HT receptor activation is the modulation of voltage-gated K^+ channels (12). In the Abelson murine leukemia virus (AMV)-transformed 18–81 pre-B-cell line, 5-HT acting through 5-HT_1- and 5-HT_3-like receptors has been reported to increase the maximum conductance and accelerate inactivation of a voltage-gated K^+ channel termed type *n* (11). Such modulation of the type *n* K^+ channel would be expected to affect lymphocyte activation, since the channel regulates the membrane potential and consequently influences the Ca^{2+} signaling response (9). The type *n* K^+ channel in the mouse, rat and human has recently been determined to be encoded by the Shaker subfamily gene, Kv1.3 (15).

In Jurkat T-cells, 5-HT has been reported to induce an increase in cytosolic Ca^{2+} by acting via 5-HT_{1c} or 5-HT_2 receptor (5). We therefore examined the effect of 5-HT, acting via the 5-HT_{1c} receptor, on Kv1.3 channels. The *Xenopus* oocyte system (28) was chosen for our experiments, since the expressed proteins, including Kv1.3 and the 5-HT_{1c} receptor, retain the properties of their native counterparts (10, 19). An added advantage of the oocyte system is its usefulness for examining the effects of channel or receptor mutants on second messenger-mediated modulation. Kv1.3 currents were suppressed by 5-HT acting through a G protein- and Ca^{2+}-dependent second messenger pathway. Another mouse T-cell voltage-gated K^+ channel, type *l*, encoded by the Shaw subfamily gene, Kv3.1 (10), was not modulated by 5-HT.

MATERIALS AND METHODS

Chemicals and solutions. The following chemicals were purchased from Sigma Chemical (St. Louis, MO): 5-HT, tricaine, guanosine 5′-*O*-(3-thiotrisphosphate) (GTPγS), phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-dibutyrate (PDBU), 4α-phorbol 12,13-dideconoate (4α-PDD), ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), calcineurin, and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonic acid (W-7). Collagenase B and alkaline phosphatase were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Staurosporine, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7), and okadaic acid were obtained from Calbiochem (San Diego, CA), and pertussis toxin (PTX) was bought from List Biological Laboratories (Campbell, CA). The ND96 buffer contained (in mM) 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 5 sodium pyruvate (pH 7.5). Mammalian Ringer solution contained (in mM) 160 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES (pH 7.4).

In vitro transcription of Kv1.3, Kv3.1, and 5-HT_{1c} receptor DNA. The mouse Kv1.3 gene was cloned into the plasmid pSP64T and the coding region of mouse Kv3.1 cDNA was cloned into pBluescript, as described earlier (14). The mouse 5-HT_{1c} receptor gene in the PGEM3 vector was a gift of N. Davidson (18). These plasmids contain the inserted gene juxtaposed to SP6, T3, or T7 promoters. The plasmids were linearized and cRNA transcribed using the appropriate polymerases, precipitated in ethanol and stored at −70°C until used. A truncated version of Kv1.3 was constructed by cleaving 367 bp of the 5′ end of the coding region using the restriction enzyme *Eag* I and subcloning into pBluescript. The truncation removes a po-

tential tyrosine kinase site at position 115 and a protein kinase (PK) A site at position 70, and this construct appears to utilize the downstream Met-147 as the translation start site (15). Truncated Kv1.3 cRNA was coinjected with 5-HT_{1c} receptor cRNA to study the role of the NH_2-terminal in 5-HT-mediated modulation on Kv1.3.

Preparation and injection of Xenopus laevis oocytes. *Xenopus laevis* frogs were anesthetized in 0.3% tricaine, and several ovarian lobes were excised. Follicular cells were removed by treatment with 0.5 U/ml collagenase in Ca^{2+}-free saline. Stage V and VI oocytes were selected and allowed to recover overnight in ND96 with supplements. Oocytes were injected with 46 nl RNA (2.3 pg for K^+ channels and 2.3 ng for 5-HT_{1c} receptor) in 0.01 M tris(hydroxymethyl)aminomethane (Tris)·HCl (pH 6.5), using a motorized positive-displacement micropipette (Drummond Scientific, Broomall, PA).

Electrophysiology. All experiments were done at room temperature (20–26°C). Two to five days after injection of the appropriate cRNA, K^+ currents were recorded using a two-electrode voltage-clamp technique (28). Cells were bathed in ND96. Electrodes were filled with 3 M KCl and had resistances of 0.5–1.0 MΩ. Only those oocytes with resting membrane potential between −40 and −70 mV were chosen for experiments. Membrane currents were recorded with a two-electrode voltage-clamp amplifier (Warner Instruments, Hamden, CT). The command input of the amplifier was controlled by an IBM-compatible computer using pClamp software (version 5.5.1, Axon Instruments, Burlingame, CA) via the TL-1 DMC Labmaster interface. Oocytes were held at −100 mV and depolarized to +40 mV at 60-s intervals for 500 ms (unless otherwise stated) to detect K^+ currents. Capacitive and leak currents were subtracted before analysis using the P/4 procedure.

For patch-clamp experiments, the vitelline membrane was mechanically removed by placing the oocytes in hypertonic solution containing 192 mM NaCl. The patch-clamp amplifier (List L/M-EPC7, Adams and List Associates, Great Neck, NY) was used in the voltage-clamp mode. Patch electrodes were pulled from Accu-fill 90 micropettes (Becton Dickinson, Parsippany, NJ) in three stages, coated with Sylgard (Dow Corning, Midland, MI), and fire polished to resistances measured of 2–6 MΩ in the bath. Electrodes were filled with mammalian Ringer in which NaCl was replaced by sodium aspartate. The bath solution contained 150 mM potassium aspartate, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, and either 0.63 or 9.86 mM $CaCl_2$, which is equivalent to free Ca^{2+} concentrations of 10^{-8} and 10^{-5} M, respectively. The command input of the patch-clamp amplifier was controlled by a PDP 11/73 computer via a digital-to-analog converter, and membrane currents were recorded at a bandwidth of 2 kHz. The holding potential was adjusted to $E = -80$ mV.

For modulation experiments, K^+ currents were allowed to stabilize for 5 min, and 5-HT was then perfused at a concentration of 100 nM in ND96. For elucidating second messenger pathways, PMA (10 nM), PDBU (100 nM), and 4α-PDD (100 nM) in ND96 were perfused into the bath solution while the oocyte was being clamped. To study the effect of PKC or calmodulin inhibitors, oocytes expressing Kv1.3 and 5-HT_{1c} receptor were incubated overnight in H-7 (500 μM), staurosporine (400 nM), or W-7 (50 μM). In other experiments, oocytes expressing Kv1.3 cRNA were injected with 46 nl of 20 mM $CaCl_2$, 50 mM EGTA, 1 μM GTPγS, 100 μg/ml PTX, calcineurin (0.5 μg/μl), or alkaline phosphatase (0.047 U) in water, or the bath solution was perfused with the phosphatase inhibitor okadaic acid (2.5 μM). Calculation of dissociation constants (K_d) for K^+ channel blockers tetraethylammonium (TEA) and charybdotoxin (CTX) were done using the formula K_d = (concn of drug)/[(1/fraction of unblocked current) − 1], assuming a 1:1 binding ratio of the drug to the channel.

RESULTS

Modulation of Kv1.3 and Kv3.1 currents by 5-HT. Membrane depolarization to +40 mV elicited outward K^+ currents in the range of 5–10 μA in oocytes coinjected with Kv1.3 and 5-HT_{1c} receptor cRNA ($n = 6$). The cloned mouse Kv1.3 channel has been shown to possess biophysical and pharmacological properties identical to those of the type *n* K^+ channel in T-cells (10). Upon perfusion of 100 nM 5-HT, there was an elevation of intracellular Ca^{2+} as evidenced by the activation of endogenous Ca^{2+}-activated Cl^- currents, which desensitized rapidly (30). The Cl^- currents were distinguished from K^+ currents by their inward tails at −100 mV, which disappeared in 1–2 min. This was followed by a progressive time-dependent reduction in the peak current amplitude of the outward Kv1.3 currents until maximal suppression was reached at ~20 min (Fig. 1*A*). The time required for suppression of half the K^+ currents ($t_{½}$) was 7 ± 0.9 min ($n = 5$). Values for $t_{½}$ represent means ± SE. Kv1.3 currents were not detectable for at least 2 h after washout of 5-HT. Control oocytes injected with Kv1.3 cRNA alone and perfused with ND96 showed a much slower rundown of K^+ currents (natural time-dependent decay of I_K) with a $t_{½} > 30$ min (Fig. 1*B*, $n = 6$); a similar Kv1.3 time-dependent rundown of Kv1.3 currents was seen in ND96-perfused oocytes coinjected with Kv1.3 and 5-HT_{1c} receptor cRNA. Control oocytes (expressing Kv1.3 alone) when perfused with 100 nM 5-HT exhibited rundown with $t_{½} > 30$ min, similar to those perfused with ND96 ($n = 5$, data not shown), implying that native oocytes do not express 5-HT receptors. In oocytes coexpressing Kv1.3 and 5-HT_{1c} receptor, the voltage dependence of activation of Kv1.3 currents and inactivation were similar in the absence or presence of 5-HT (Fig 1, *A* and *C*). These results are surprising, since 5-HT has been reported to increase maximum conductance and accelerate inactivation of type *n* K^+ channels in B-cells (11).

We also examined the effect of 5-HT on another T-cell voltage-gated K^+ channel, type *l*, encoded by the Shaw-related Kv3.1 gene (10). Robust K^+ currents were detected in oocytes coinjected with 5-HT_{1c} receptor and Kv3.1 cRNA (data not shown). The Kv3.1 currents decayed with a $t_{½} > 30$ min ($n = 6$) in oocytes perfused with ND96 (Fig. 1*B*). After the addition of 5-HT, Kv3.1 currents decayed with a similar $t_{½} > 30$ min ($n = 6$), a time frame consistent with rundown (Fig. 1*B*). Figure 1*D* shows that the voltage dependence of activation and the peak current amplitude of Kv3.1 currents were similar in the presence or absence of 5-HT.

We next set out to elucidate the second messenger systems that couple 5-HT_{1c} receptor to the Kv1.3 channel.

Involvement of G proteins. In oocytes, 5-HT_{1c} receptor is coupled to the Ca^{2+} signaling pathway through a PTX-sensitive G protein (13). To study the involvement of G proteins in the 5-HT-mediated inhibition of Kv1.3 currents, we injected the nonhydrolyzable GTP analogue, GTPγS, into oocytes expressing Kv1.3 ($n = 4$). GTPγS suppressed the Kv1.3 K^+ currents with a $t_{½}$ of 8 ± 0.85 min (Fig. 2*A*) reminiscent of 5-HT-mediated K^+ channel inhibition. Second, injection of PTX (4.6 μg), a G protein inhibitor, prevented the 5-HT-mediated modulation of

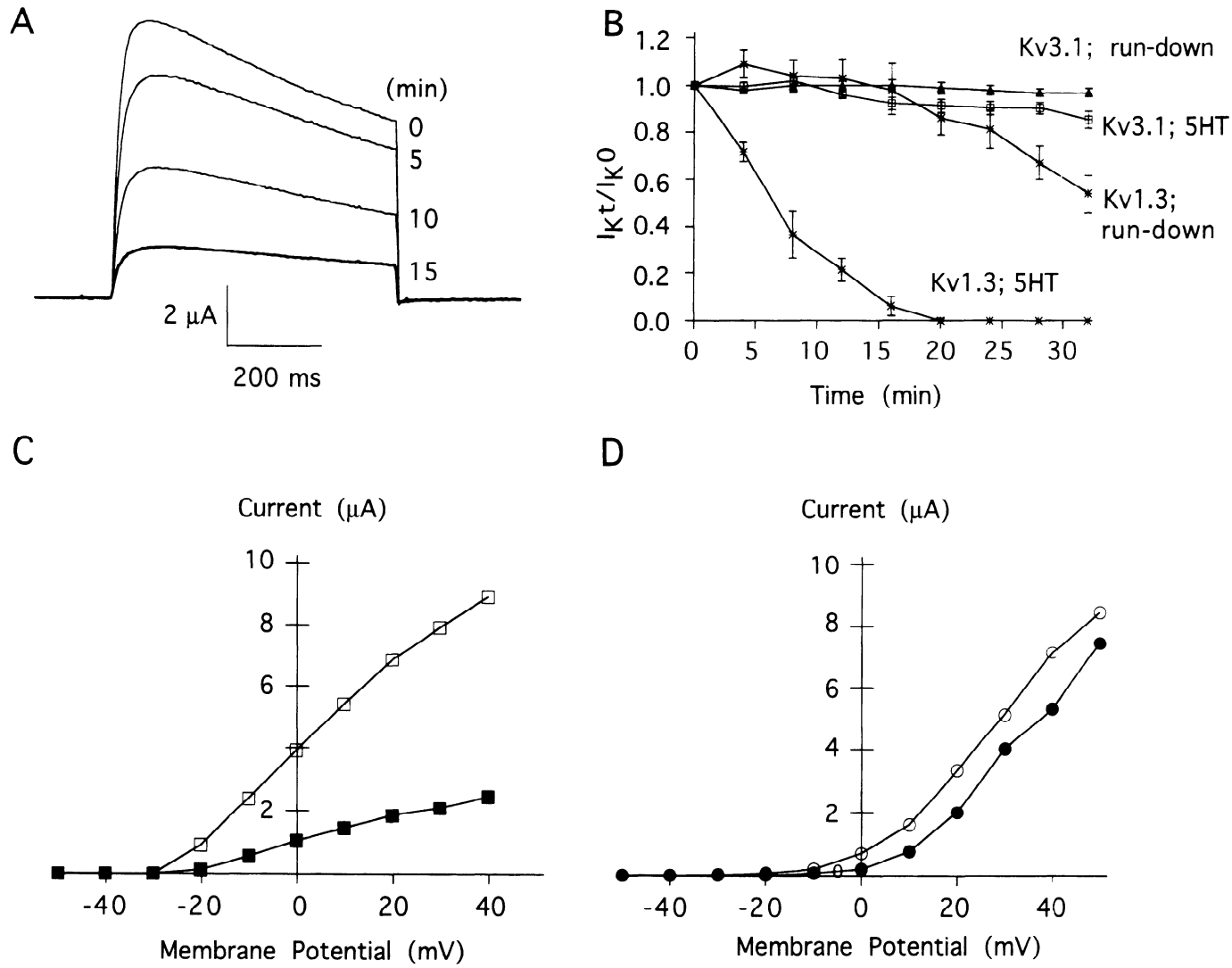

Fig. 1. Serotonin (5-hydroxytryptamine, 5-HT) mediated suppression of Kv1.3 currents but not Kv3.1 currents heterologously expressed in oocytes along with 5-HT_{1c} receptor cRNA. *A*: effect of 100 nM 5-HT on Kv1.3 currents. *B*: time course of Kv1.3 suppression by 5-HT compared with Kv1.3 rundown. Also compared is decay of Kv3.1 currents in presence or absence of 5-HT. For rundown experiments, oocytes injected with Kv1.3 or Kv3.1 cRNA alone were perfused with ND96 and slow time-dependent decay of K^+ current (I_K) measured. Data points represent means ± SE of ratio of current at *time t* ($I_K t$) to current at *time 0* ($I_K 0$). *C*: current-voltage (*I-V*) curve of Kv1.3 channels before (□) and after (■) addition of 5-HT. Data points for *I-V* curve were sampled 2 min after application of 5-HT. Because suppression of Kv1.3 currents was ongoing during generation of *I-V* curve (10 steps, pulsed at 60-s intervals), all data points were normalized to the one after 10 min of 5-HT application, using time course of 5-HT-mediated modulation (see *B*). *D*: *I-V* curve of Kv3.1 channels before (○) and 30 min after (●) addition of 100 nM 5-HT.

Kv1.3 currents (Fig. 2*A*). The minimal reduction in Kv1.3 currents observed at $t = 20$ min in PTX-treated oocytes (Fig. 2*A*) is consistent with rundown (cf. Fig. 1*B*). Collectively, these results suggest that a PTX-sensitive G protein is involved in the second messenger pathway coupling 5-HT_{1c} receptor to the Kv1.3 channel in oocytes.

Ca^{2+} as a second messenger. The next step in the 5-HT_{1c} receptor-triggered cascade is the induction of inositol 1,4,5-trisphosphate (IP_3) and the consequent release of Ca^{2+} from intracellular stores (19). To ascertain whether Ca^{2+} is necessary for 5-HT-mediated modulation, three oocytes coexpressing Kv1.3 and 5-HT_{1c} receptor were injected with the Ca^{2+} chelator, EGTA (46 nl of 50 mM), and bathed in Ca^{2+}-free ND96 solution. EGTA prevented the induction of Ca^{2+}-dependent Cl^- currents and abolished 5-HT-mediated suppression of Kv1.3 currents in all three oocytes (Fig. 2*B*). In addition, perfusion of Kv1.3-expressing oocytes with fetal calf serum (1:1,000), which has been previously shown to induce the IP_3 and Ca^{2+} signaling response in oocytes (23), caused suppression of Kv1.3 currents with a time course remarkably similar to that of 5-HT (data not shown). To further test the involvement of Ca^{2+}, oocytes expressing Kv1.3 channels were injected with 46 nl of 20 mM $CaCl_2$ ~1 min before voltage-clamp recording. A slow submaximal reduction of Kv1.3 currents was observed in three oocytes with an average $t_{1/2} = 15 \pm 1.15$ min (Fig. 2*B*). This was probably due to slow diffusion of Ca^{2+} from the site of injection to other regions of the oocyte, combined with buffering of Ca^{2+} in the cytosol.

Ca^{2+} does not directly block Kv1.3 channel. To deter-

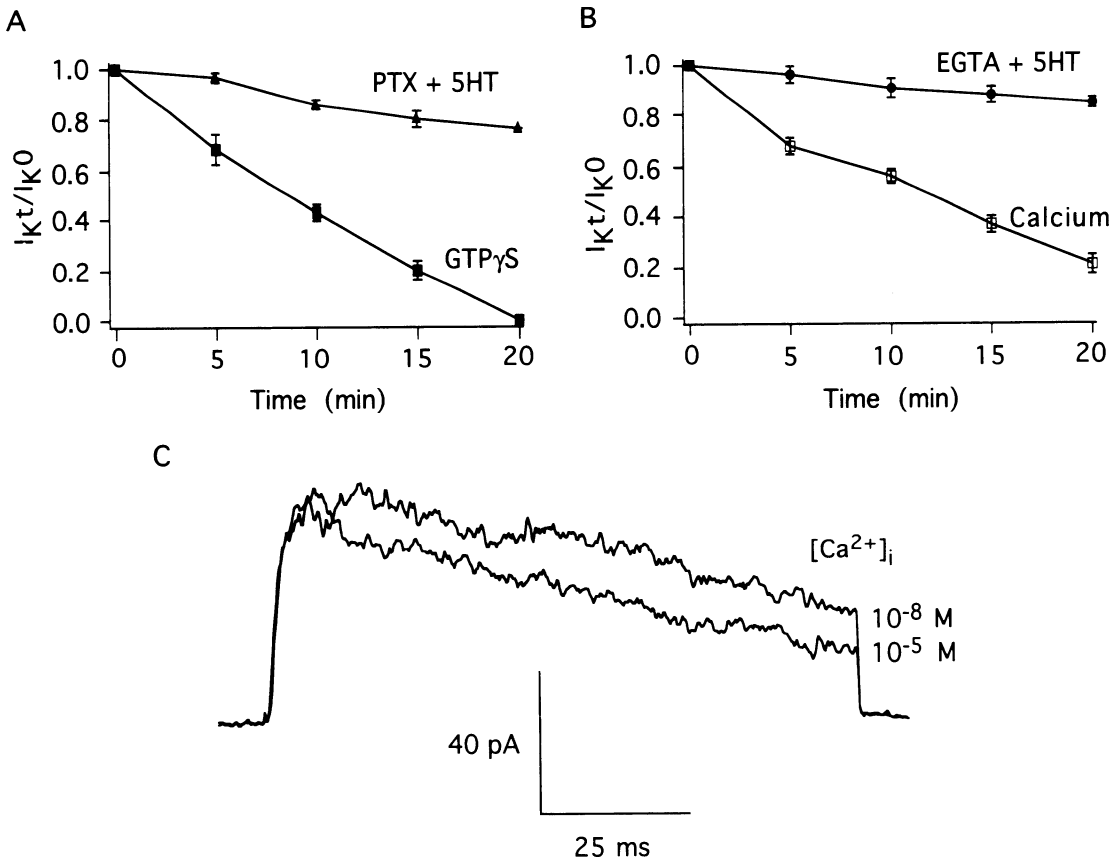

Fig. 2. Activation of a pertussin toxin (PTX)-sensitive G protein and rise in intracellular Ca^{2+} are necessary for 5-HT-mediated modulation of Kv1.3, but Ca^{2+} does not directly modulate Kv1.3 currents. *A*: guanosine 5′-*O*-(3-thiotrisphosphate) (GTPγS, 1 μM) suppresses Kv1.3 currents with a time frame similar to that mediated by 5-HT. It is also shown that PTX (4.6 μg), when injected into oocytes (5 min before start of recording) coexpressing Kv1.3 and 5-HT_{1c}cRNA, prevents suppression of Kv1.3 currents by 5-HT (100 nM perfused at *time 0*). Data points represent means ± SE of ratio of $I_K t$ to $I_K 0$. *B*: $CaCl_2$ (46 nl of 20 mM) when injected into oocytes expressing Kv1.3 alone suppressed Kv1.3 currents with a slower time course than caused by 5-HT (see Fig. 1*B*). Traces were recorded ~1 min after injection of $CaCl_2$. It is also shown that EGTA abolishes 5-HT-mediated suppression of Kv1.3 currents. EGTA (46 nl of 50 mM) was injected into oocytes at *time 0*, and 5-HT was added 1 min later. *C*: application of $CaCl_2$ (10 μM) to an inside-out patch of membrane expressing Kv1.3 channels altered inactivation of channel but did not suppress peak current amplitude.

mine whether Ca^{2+} directly blocked the Kv1.3 channels, we isolated inside-out patches of oocytes containing Kv1.3 channels and treated these with 10 μM Ca^{2+}. The peak current amplitude was not reduced, although inactivation was accelerated (Fig. 2*C*). These results confirm earlier studies on the native type *n* K^+ channel in T-cells, which demonstrated that intracellular Ca^{2+} at micromolar concentrations (levels reached during a normal activation response) increased channel inactivation (8, 14), probably by interacting with a binding site within the Kv1.3 pore. These results, along with those stated earlier, indicate that suppression of Kv1.3 currents is not due to a direct effect of Ca^{2+} but is instead a consequence of activation of other second messengers.

5-HT-mediated suppression of Kv1.3 currents is not due to phosphorylation of protein by PKC. Activation of the 5-HT_{1c} receptor second messenger pathway would be expected to trigger Ca^{2+}- and diacylglycerol-dependent PKC. Potential sites for phosphorylation by PKC (16) are present at positions 343 and 347 in the internal loop linking the S4 and S5 transmembrane segments of the Kv1.3 protein (15). We therefore examined the effects of two PKC activators, PMA (10 nM) and PDBU (100 nM), on Kv1.3 currents. Both PMA and PDBU blocked Kv1.3 currents in a time-dependent manner with a $t_{1/2}$ of 13 ± 1.05 and 14 ± 1.33 min, respectively ($n = 6$ each, Fig. 3, *A-C*). The structurally related but inactive phorbol ester, 4-αPDD, did not suppress Kv1.3 currents in four oocytes ($t_{1/2} > 30$ min, Fig. 3*C*). Furthermore, the PKC inhibitors, H-7 and staurosporine, prevented the phorbol ester-mediated suppression of Kv1.3 currents (Fig. 3*D*), suggesting that the effects of PMA and PDBU on Kv1.3 were due to PKC activation. However, in the presence of 500 μM H-7 and 400 nM staurosporine (Fig. 3*D*), 5-HT caused Kv1.3 currents to decay, with a $t_{1/2} = 8 \pm 0.7$ min ($n = 4$ each), a time frame identical to the 5-HT-mediated Kv1.3 suppression in the absence of these drugs ($t_{1/2} = 7 \pm 0.9$ min). We conclude that phorbol esters and 5-HT both modulate Kv1.3 currents but by independent pathways.

Phosphorylation by tyrosine kinase and calmodulin-dependent kinase is not part of chain of events connecting 5-HT_{1c}R to the Kv1.3 channel. A potential site for phosphorylation (*R*PSF*D*AIL*YYY*) by tyrosine kinase (16) is present at position 114 in the NH_2-terminal of Kv1.3

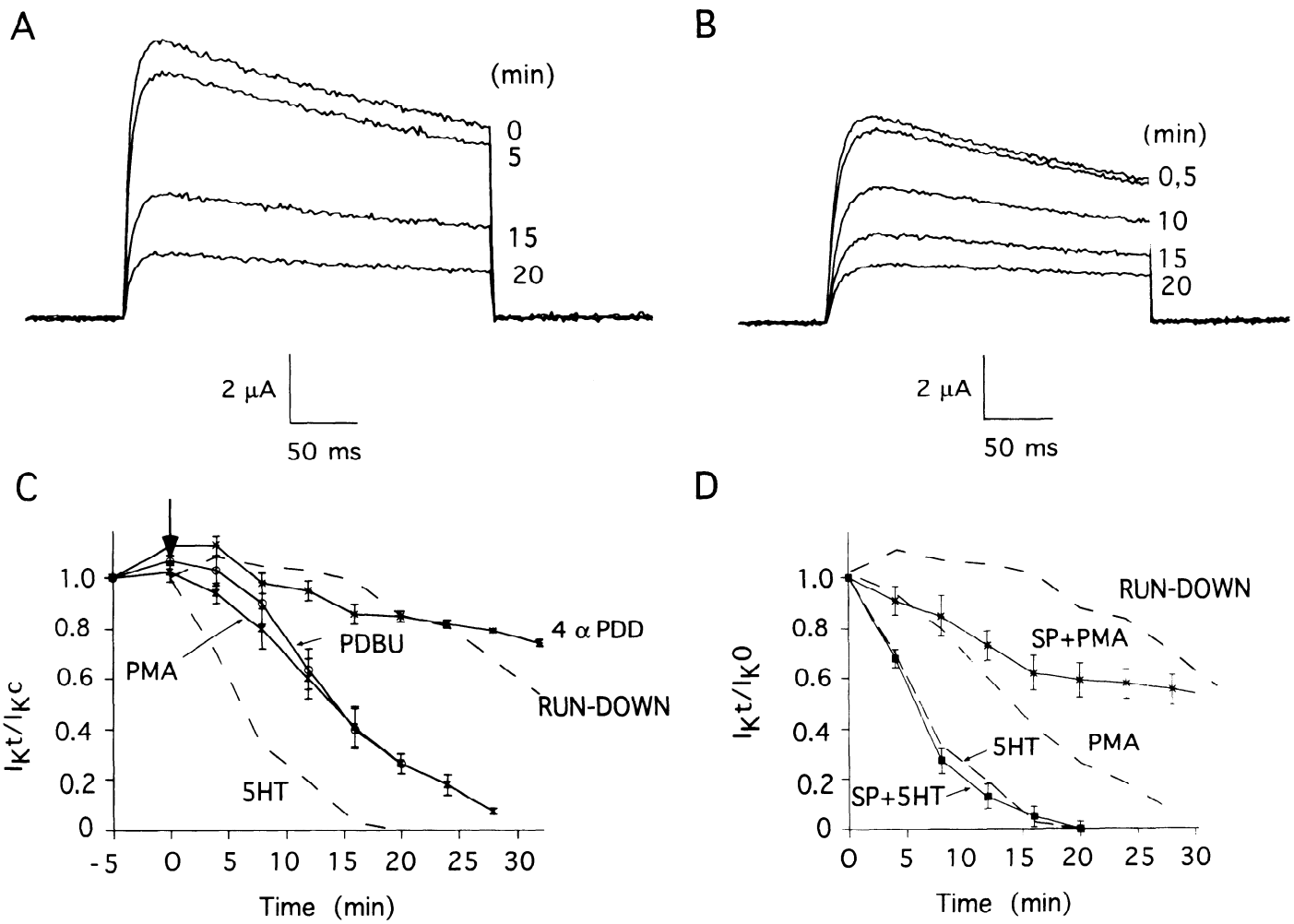

Fig. 3. Phorbol esters suppress Kv1.3 currents. Effect of 10 nM phorbol 12-myristate 13-acetate (PMA, *A*) and 100 nM phorbol 12,13-dibutyrate (PDBU, *B*) on Kv1.3 currents in oocytes injected with Kv1.3 cRNA alone. *C*: time course of Kv1.3 modulation by both phorbol esters is compared with that of inactive analogue, 4α-phorbol 12,13-dideconoate (4α-PDD). Dashed lines represent time-dependent decay of Kv1.3 currents in presence of 5-HT (100 nM) or ND96 buffer (rundown). Data represent means ± SE of ratio of $I_K t$ to current at *time c* ($I_K c$). *D*: PKC inhibitor, staurosporine (SP), prevented PMA-mediated suppression of Kv1.3 currents (SP + PMA) but had no effect on 5-HT-dependent suppression. Dashed lines represent time-dependent decay of Kv1.3 currents in presence of 5-HT (100 nM) or PMA (10 nM). SP + PMA time-dependent decay of Kv1.3 currents is similar to Kv1.3 rundown shown in Fig. 3*C*. Data represent means ± SE of ratio of $I_K t$ to $I_K c$.

(15), and tyrosine phosphorylation might link 5-HT_{1c} receptor to the Kv1.3 channel. To address this possibility, we coexpressed 5-HT_{1c} receptor along with a truncated Kv1.3 gene product (initiating at Met-147) in oocytes and examined the effect of 5-HT on K^+ currents. The deletion removed the putative tyrosine phosphorylation site as well as a consensus sequence for phosphorylation by adenosine 3′,5′-cyclic monophosphate-dependent PKA (position 70) but no putative PKC sites. The low level of expression of the truncated gene product (tKv1.3) in oocytes precluded patch-clamp measurements of these K^+ currents. We therefore compared the electrophysiological properties of the full-length and truncated Kv1.3 gene products by the two voltage-clamp method. The biophysical properties of the two channels were qualitatively similar; they activated at approximately −30 mV and were half blocked by 20 mM external TEA and by charybdotoxin (e.g., Fig. 4*A*; tKv1.3, $K_d = 5.53 \pm 0.28$; Kv1.3, $K_d = 7.12 \pm 0.35$; $n = 4$ in each case). These results with the two microelectrode clamps differ from our earlier patch-clamp results (10) and probably reflect previously described quantitative differences between the two methods. In the presence of 5-HT (100 nM), the truncated Kv1.3 K^+ currents were suppressed with a $t_{1/2} = 8 \pm 0.35$ min, comparable with full-length Kv1.3 (Fig. 4*B*), indicating that the first 146 residues in the NH_2-terminal, containing putative tyrosine kinase and PKA phosphorylation sites (but lacking PKC phosphorylation sites), are not necessary for 5-HT-mediated suppression of Kv1.3 currents. As expected, the truncated channel was modulated by PKC activators PMA and PDBU with time courses similar to modulation of the full-length channel (data not shown).

Calmodulin activation is an important consequence of the Ca^{2+} signaling response. The activated Ca^{2+}-binding protein can then initiate phosphorylation or dephosphorylation of proteins by calmodulin-dependent kinases and phosphatases. The Kv1.3 channel is a potential tar-

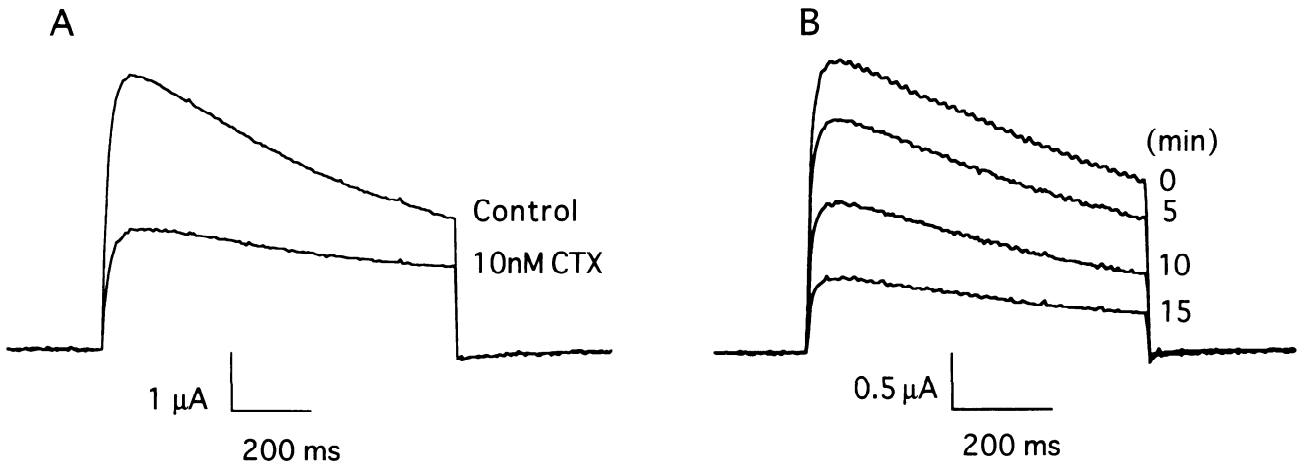

Fig. 4. First 146 amino acids in NH_2-terminal are not required for 5-HT-mediated modulation of Kv1.3. *A*: K^+ currents in oocytes injected with truncated Kv1.3 cRNA and examined by two microelectrode clamp method. Currents are blocked by 10 nM charybdotoxin (CTX; K_d = ~5 nM) like full-length Kv1.3 channels examined under similar conditions. *B*: K^+ currents in oocytes injected with 5-HT_{1c} receptor and truncated Kv1.3 cRNA, after addition of 5-HT (100 nM). 5-HT suppresses truncated Kv1.3 channels (lacking potential NH_2-terminal tyrosine kinase and PKA consensus sites) with a similar time course as full-length Kv1.3 channel protein.

get for modulation by this second messenger, since a consensus sequence for phosphorylation by a multifunctional calmodulin-dependent kinase (16) is present at position 475 in the COOH-terminal of the protein (15). Oocytes coexpressing 5-HT_{1c} receptor and Kv1.3 were therefore incubated for 60 min with W-7 (50 μM), a calmodulin antagonist. W-7 treatment did not prevent 5-HT-mediated suppression of Kv1.3 currents ($t_{1/2}$ = 7 ± 0.23 min; n = 3).

5-HT does not inhibit Kv1.3 currents by dephosphorylating protein. Protein phosphatases, activated by the 5-HT_{1c} receptor-dependent second messenger cascade, could dephosphorylate the Kv1.3 protein and thereby block the channel. Two types of experiments were performed to test this possibility. First, application of alkaline phosphatase (0.047 U) or calcineurin (0.5 μg/ml) into oocyte patches expressing Kv1.3 channels did not suppress Kv1.3 currents ($t_{1/2}$ > 30 min, n = 2). Second, the protein phosphatase inhibitor, okadaic acid (2.5 μM), did not prevent 5-HT-induced inhibition of Kv1.3 currents ($t_{1/2}$ = 7 ± 0.85, n = 2). Thus suppression of Kv1.3 currents does not appear to be a result of protein dephosphorylation.

DISCUSSION

The *Xenopus* oocyte heterologous expression system was used to define the molecular mechanisms linking 5-HT_{1c} receptor to the mouse T-cell K^+ channel, type *n*, which is encoded by the Kv1.3 gene (10). 5-HT, acting through the 5-HT_{1c} receptor, suppressed Kv1.3 currents for a sustained period but had no effect on the channel encoded by the Shaw subfamily gene, Kv3.1. The nonhydrolyzable GTP analogue, GTPγS, mimicked this effect, whereas the G protein inhibitor, PTX, prevented suppression of the Kv1.3 channel by 5-HT. Previous studies have shown that voltage-gated ion channels are modulated in a variety of ways by receptor-mediated G protein activation (3).

5-HT-mediated suppression of Kv1.3 currents was abolished by EGTA. A critical role for Ca^{2+} was further supported by the demonstration that intracellular injection of Ca^{2+} suppressed Kv1.3. Because Ca^{2+} did not directly block the channel, we focused our efforts on identifying the Ca^{2+}-dependent second messenger systems responsible for Kv1.3 modulation. PKC activation was not part of the cascade coupling 5-HT_{1c} receptor to the Kv1.3 channel, since inhibitors of PKC had no effect on 5-HT-mediated modulation. Tyrosine phosphorylation, calmodulin activation, and phosphatase-mediated dephosphorylation of Kv1.3 were also not required for 5-HT-mediated suppression of the Kv1.3 channel. Thus G protein activation and the Ca^{2+} signaling response are critical components of the second messenger cascade connecting 5-HT_{1c} receptor to Kv1.3, but the terminal steps in this pathway remain undetermined. Our results are similar to those obtained on other closely related potassium channels modulated by 5-HT_{1c} or 5-HT_2 receptors (4, 20).

Recently, the cloned mouse brain K^+ channel Kv1.1 (MBK1), was shown to be modulated by 5-HT, although suppression was only partial and transient (18). The greater susceptibility of Kv1.3 to complete and sustained blockade by 5-HT, must depend on the sequence differences between these two closely related proteins. Unlike Kv1.3, 5-HT-mediated inhibition of Kv1.1 was dependent on calmodulin activation, since antagonists of this protein prevented modulation of the channel. In a second report, Panicker and colleagues (23) demonstrated that 5-HT, acting through 5-HT_{1c} receptor, suppressed rat cortical K^+ channels, expressed heterologously in oocytes, in a Ca^{2+}-independent manner. These studies suggest that 5-HT_{1c} receptor modulates different K^+ channels through distinct second messenger pathways.

We have shown that PKC activators are capable of independently suppressing Kv1.3 currents and that this effect is reversed by PKC inhibitors. Recently, the type *n* K^+ channel in human Jurkat T-cells has been shown to be suppressed by protein kinases A and C (24). Our results on the cloned Kv1.3 channel using a heterologous

expression system are consistent with the findings in T-cells. Mouse, rat, and human Kv1.3 have a putative PKC phosphorylation site (TLKA*S*MR) in the intracellular loop linking the S4 and S5 transmembrane segments (15). Rat Kv1.3, translated in vitro, has been reported to be phosphorylated by PKC (Y. C. Cai and J. Douglass, personal communication), presumably at this serine which is one of about six residues known to affect the single K^+ channel conductance and thought to line the channel vestibule (26). Thus phosphorylation of this site might suppress mouse Kv1.3 currents by altering the ion conduction pathway.

A truncated Kv1.3 gene product starting at Met-147 produced functional K^+ currents that were qualitatively similar to those generated by the full-length Kv1.3 polypeptide (10). The deletion removes putative tyrosine kinase- and PKA-phosphorylation sites but no PKC sites. The truncated K^+ channel was modulated by 5-HT and by phorbol esters with time courses that were remarkably similar to that of full-length Kv1.3. Interestingly, the truncated channel lacks the motif that has been suggested to be necessary for subunit assembly of Shaker K^+ channels (21). Our results imply that regions other than the NH_2-terminal are involved in channel assembly.

In the AMV-transformed pre-B-cell line, 18-81, 5-HT (10-20 μM) acting via 5-HT_1-like receptors was reported to enhance type *n* K^+ conductance, whereas activation of 5-HT_3-like receptors by 5-HT was shown to accelerate inactivation (11). The apparent inconsistency between our results and those of Choquet and Korn (11) may be accounted for by differences in the 5-HT receptors expressed in AMV-transformed pre-B-cells compared with the 5-HT_{1c} receptor used in the present study. We chose to study the effect of 5-HT_{1c} receptor on type *n* K^+ channels because a similar receptor has been detected in Jurkat T-cells (5). Even if 5-HT_{1c} receptor is expressed in the AMV-transformed pre-B-cells, the use of whole cell recording techniques with pipette solutions buffering internal Ca^{2+} would have excluded Ca^{2+}-dependent 5-HT_{1c} receptor-mediated effects on type *n* K^+ channels (9). The buffering of internal Ca^{2+} may also explain why a dose of 5-HT (100 nM) that would be expected to activate 5-HT_{1c} receptor did not modulate type *n* K^+ channels in the AMV-transformed pre-B-cells (11).

In conclusion, by exploiting the *Xenopus* oocyte expression system, we have demonstrated 5-HT- and PKC-mediated modulation of the T-cell K^+ channel, Kv1.3. Because Kv1.3 mRNA expression has also been detected in the brain (6), which is rich in 5-HT_{1c} receptor, 5-HT could modify neuronal behavior by modulating Kv1.3 channels. Site-specific mutagenesis strategies, coupled with the cell physiological approaches we have used, could be used in future to define those sites on Kv1.3 that are critical for 5-HT-mediated modulation. Substance P and β-adrenergic agonists also suppress type *n* K^+ currents (25, 27). Thus modulation of K^+ channel activity in lymphocytes by a variety of neurotransmitters may be a major factor in controlling the lymphocyte activation response.

We thank Michael Strong for generating the truncated Kv1.3 construct, George Gutman, Robert Spencer, Grischa Chandy, and Michael Cahalan for useful and insightful comments, and Alan Goldin for help with the *Xenopus* oocyte heterologous expression system.

This study was supported by a grant from Pfizer (Groton, CT), National Institute of Allergy and Infectious Diseases Grant AI-24783, and Postdoctoral Fellowship 92-59 from the California Chapter, American Heart Association.

Address for reprint requests: J. Aiyar, Medical Sciences Bldg. I, Dept. of Physiology and Biophysics, Univ. of California, Irvine, CA 92717.

Received 8 March 1993; accepted in final form 24 June 1993.

REFERENCES

1. **Ader, R., D. Felton., and N. Cohen.** Interactions between the brain and the immune system. *Annu. Rev. Pharmacol. Toxicol.* 30: 561-602, 1990.
2. **Ameisen, J., R. Meade, and P. W. Askenase.** A new interpretation of the involvement of serotonin in delayed-type hypersensitivity. Serotonin-2 receptor antagonists inhibit contact sensitivity by an effect on T cells. *J. Immunol.* 142: 3171-3179, 1989.
3. **Armstrong, D. L., and R. E. White.** An enzymatic mechanism for potassium channel stimulation through pertussis-toxin-sensitive G proteins. *Trends Neurosci.* 15: 403-408, 1992.
4. **Attali, B., E. Honoré, M. Lazdunski, and J. Barhanin.** Regulation of a major cloned voltage-gated K^+ channel from human T lymphocytes. *FEBS Lett.* 303: 229-232, 1992.
5. **Aune, T. M., K. A. Kelly, G. E. Ranges, and M. P. Bombara.** Serotonin-activated signal transduction via serotonin receptors on Jurkat cells. *J. Immunol.* 145: 1826-1831, 1990.
6. **Beckh, S., and O. Pongs.** Members of the RCK potassium channel family are differentially expressed in the rat nervous system. *EMBO J.* 9: 777-782, 1990.
7. **Bonnet, M., G. Lespinats, and C. Burtin.** Histamine and serotonin suppression of lymphocyte response to phytohemagglutinin and allogeneic cells. *Cell. Immunol.* 83: 280-291, 1984.
8. **Bregestovski, P., A. Redkozubov, and A. Alexeev.** Elevation of intracellular calcium reduces voltage-dependent potassium conductance in human T cells. *Nature Lond.* 319: 776-779, 1986.
9. **Cahalan, M. D., K. G. Chandy, and S. Grissmer.** Potassium channels in development, activation and disease in T lymphocytes. *Curr. Topics Membr.* 39: 357-393, 1991.
10. **Chandy, K. G., G. A. Gutman, and S. Grissmer.** Physiological role, molecular structure and evolutionary relationships of voltage-gated potassium channels in T lymphocytes. *Semin. Neurosci.* 5: 125-134, 1993.
11. **Choquet, D., and H. Korn.** Dual effects of serotonin on a voltage-gated conductance in lymphocytes. *Proc. Natl. Acad. Sci. USA* 85: 4557-4561, 1988.
12. **Colino, A., and J. V. Halliwell.** Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature Lond.* 328: 73-77, 1989.
13. **Dascal, N., C. Ifune, R. Hopkins, T. P. Snuch, H. Lubbert, N. Davidson, and H. A. Lester.** Involvement of a GTP-binding protein in mediation of serotonin and acetylcholine responses in *Xenopus* oocytes injected with rat brain messenger RNA. *Mol. Brain. Res.* 78: 1411, 1986.
14. **Grissmer, S., and M. D. Cahalan.** Divalent ion trapping inside potassium channels of human T lymphocytes. *J. Gen. Physiol.* 93: 609-630, 1986.
15. **Gutman, G. A., and K. G. Chandy.** Nomenclature of mammalian voltage-dependent potassium channel genes. *Semin. Neurosci.* 5: 101-106, 1993.
16. **Hanks, S. K., and A. M. Quinn.** Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. *Methods Enzymol.* 200: 38-62, 1991.
17. **Hellstrand, K., and S. Hermodsson.** Role of serotonin in the regulation of human natural killer cell cytotoxicity. *J. Immunol.* 139: 869-875, 1987.
18. **Hoger, J. H., A. E. Walter, D. Vance, L. Yu, H. A. Lester, and N. Davidson.** Modulation of a cloned mouse brain potassium channel. *Neuron* 6: 227-236, 1991.
19. **Julius, D.** Molecular biology of serotonin receptors. *Annu. Rev. Neurosci.* 14: 335-360, 1991.
20. **Kavanaugh, M. P., M. J.Christie, P. B. Osborne, A. E. Busch, Z. Shen, Y. Wu, P. E. Seeburg, J. P. Adelman, and**

R. A. North. Transmitter regulation of voltage-dependent K^+ channels expressed in *Xenopus* oocytes. *Biochem. J.* 277: 899–902, 1991.

21. **Li, M., Y. N. Jan, and L. Y. Jan.** Specification of subunit assembly by the hydrophilic amino-terminal domain of the shaker potassium channel. *Science Wash. DC* 257: 1225–1230, 1992.
22. **Maricq, A. V., A. S. Peterson, A. J. Brake, R. M. Myers, and D. Julius.** Primary structure and functional expression of the $5HT_3$ receptor, a serotonin-gated ion channel. *Science Wash. DC* 254: 432–436, 1991.
23. **Panicker, M. M., I. Parker, and R. Miledi.** Receptors of the serotonin 1C subtype expressed from cloned DNA mediate the closing of K^+ membrane channels encoded by brain mRNA. *Proc. Natl. Acad. Sci. USA* 88: 2560–2562, 1991.
24. **Payet, M. D., and G. Dupuis.** Dual regulation of the *n*-type K^+ channel in Jurkat T-lymphocytes by protein kinase-A and kinase-C. *J. Biol. Chem.* 267: 18270–18273, 1992.
25. **Schumann, M. A., and P. Gardner.** Modulation of membrane K^+ conductance in T-lymphocytes by substance P via a GTP-binding protein. *J. Membr. Biol.* 111: 133–139, 1989.
26. **Slesinger, P. A., E. Y. Isacoff, Y. N. Jan, and L. Y. Jan.** Effect of mutations in Shaker S4-S5 cytoplasmic loop and S5 segments on ion selectivity. *Soc. Neurosci. Abstr.* 18: 921, 1992.
27. **Soliven, B., and D. J. Nelson.** Beta-adrenergic modulation of K^+ current in human T lymphocytes. *J. Membr. Biol.* 117: 263–274, 1990.
28. **Soreq, H., and S. Seidman.** *Xenopus* oocyte microinjection: from gene to protein. *Methods Enzymol.* 207: 225–265, 1992.
29. **Sternberg, M. E., J. Trial, and C. W. Parker.** Effect of serotonin on murine macrophages: suppression of Ia expression by serotonin and its reversal by $5HT_2$ serotonergic receptor antagonists. *J. Immunol.* 137: 276–282, 1986.
30. **Walter, A. E., J. H. Hoger, C. Labarca, L. Yu, and N. Davidson.** Low molecular weight mRNA encodes a protein that controls serotonin $5\text{-}HT_{1c}$ and acetylcholine M_1 receptor sensitivity in *Xenopus* oocytes. *J. Gen. Physiol.* 98: 399–417, 1991.