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Suppression of neuronal potassium A-current by arachidonic acid

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The effect of arachidonic acid on the A current (I_A) has been studied in dissociated bullfrog neurons under whole-cell voltage-clamp conditions. Arachidonic acid reduced I_A in a dose-dependent and reversible manner without a shift in the prepulse inactivation voltage–current relation. 1.75 μM inhibited I_A by 50%, and higher concentrations caused a total suppression. In addition, arachidonic acid increased the M-current (I_M), a different potassium current that does not inactivate. Neither indomethacin nor nordihydroguaiaretic acid, cyclooxygenase and lipoxygenase inhibitors respectively, prevented I_A reduction. In contrast, nordihydroguaiaretic acid prevented I_M enhancement. Eicosatetraynoic acid, an arachidonic acid analog that cannot be metabolized, also reduced I_A. These results suggest that arachidonic acid metabolism is not required to suppress I_A.

A-current; M-current; Potassium current; Inhibition; Arachidonic acid; Sympathetic neuron

1. INTRODUCTION

Modulation of ionic channels plays a fundamental role in controlling the activity of both excitable and non-excitable cells [1,2]. In the last few years, it has become clear that arachidonic acid and its metabolites should be included in the list of second messengers involved in ion channel modulation [3,4]. Arachidonic acid is produced in normal conditions in response to hormones or neurotransmitters, or in abnormal conditions such as ischemia, epilepsy or stroke [5,6]. Upon liberation from membranes (by a GTP-binding protein and/or calcium mediated stimulation of phospholipase A_2 [7], or sequential participation of phospholipase C and phospholipase D or diglyceride lipase [8]), arachidonic acid can exert its action directly on the channel (or a closely associated component) [3], or indirectly through its numerous metabolic products, including 12 and 5 lipoxygenase metabolites [9–11], or through activation of protein kinase C and oxygen radical production [12].

The A-current (I_A) is a transient voltage-dependent potassium current that has been implicated in control of cell excitability [13]. Several neurotransmitters modulate I_A in neurons [14–18] and other excitable cells [19–22]. The second messenger system involved in these responses has not been identified yet. It has been suggested, based on the action of phorbol esters, that the reduction of the transient outward current in rat ventricular myocytes may be mediated by protein kinase C [19]. However, this is in sharp contrast to the results of Tohse et al. [20] who did not observe a significant reduction in the response to phorbol esters in the same preparation, and to the augmentation observed in the response to phorbol myristate in rabbit atrial myocytes [23]. In addition, in hippocampal neurons, high concentrations of phorbol dibutyrate do not affect I_A [24].

Here, blockade of the A-current by arachidonic acid in bullfrog sympathetic neurons is described. Arachidonic acid reduces I_A at low concentrations, in a dose-dependent and reversible manner, indicating that arachidonic acid is a plausible second messenger for neurotransmitter mediated I_A modulation.

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2. EXPERIMENTAL

Bullfrog sympathetic neurons were isolated from lumbar VIIIth, IXth and Xth ganglia by enzymatic treatment and trituration as previously described [25] and used after 1–7 days in culture. Currents were recorded at room temperature (22–24°C) in continuous whole-cell voltage-clamp mode using a List EPC7 amplifier. 70–80% series resistance was compensated. Fire-polished, filamented hard glass electrodes, with a tip diameter of ~3.3 μm, were used. The total access resistance varied from 2 to 10 MΩ. The intracellular solution was 90 mM K aspartate, 20 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM Na_2ATP, 5 mM HEPES, 0.2 mM EGTA, 60 μM $CaCl_2$ (80 nM estimated free calcium), and 0.2 mM Na_3GTP. The pH was adjusted to 7.2. Neurons were constantly perfused with extracellular solution, which was 115 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$ 5 mM HEPES and 10 mM glucose (pH 7.2). To reduce contamination by other currents, 2 mM $MnCl_2$ (a calcium channel blocker), 1 mM TEA (a potassium channel blocker), 0.2 mM d-tubocurarine (a blocker of some calcium activated potassium channels) and 200 nM TTX (a sodium channel blocker) was added. This solution caused a 16 mV junction potential that was subtracted in the voltages reported. A suction pipette ~150 μm in diameter was positioned ~150 μm from the cell to exchange solutions around the neuron studied.

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Arachidonic acid was from Calbiochem (San Diego, CA), eicosatetraynoic acid (ETYA) was from Biomol (Plymouth Meeting, PA) and the rest of the chemicals were from Sigma (St Louis, MO). Arachidonic acid, ETYA, indomethacin and nordihydroguaiaretic acid were prepared from a stock solution in dimethyl sulfoxide (DMSO), to a final DMSO concentration of less than 0.25%. At this concentration, DMSO did not have an appreciable effect on the M- or A-currents. DMSO was dehydrated with 'molecular sieves' (8–12 mesh size, 3 Angstroms nominal diameter) (Fisher, Fair Lawn, NJ). Stock solutions (100 mM) of arachidonic acid and ETYA were kept at −80°C in sealed amber vials under nitrogen.

Currents were digitized and acquired on line via a Data Translation DT2801A interface (4096 current levels) and stored in an IBM compatible computer, using software written in ASYST language (ASYST 1.53; Macmillan Software Company, NY). Currents were filtered with an 8 pole Bessel filter (Frequency devices, model 902) at 1/2 the acquisition frequency (typically between 200 and 500 Hz).

Analysis was done using Sigmaplot 4.1 (Jandel Co., Corta Madera, CA). Results are expressed as mean ± S.D. (n = number of experiments).

3. RESULTS

Fig. 1A shows the protocol used to reveal the A-current (I_A). The membrane potential was held at −40 mV. At this holding potential the M-current (I_M) is the predominant current because it does not inactivate [26]. An inward current relaxation (reflecting the closure of M-channels) was evoked by a 1 s voltage step to −60 mV. When the potential was returned to −40 mV, an outward current relaxation was revealed as M-channels reopened. Increasing 1 s voltage steps were imposed 1 s after the first voltage jump. When the second voltage step was more negative than −70 mV the M-current activation relaxations were superimposed on the fast inactivating A-current, which increased as the hyperpolarizing voltage pulse removed more inactivation. The current during the first repolarization was subtracted from the current during the second repolarization to isolate I_A (Fig. 1B). This protocol caused an underestimation of the size of I_A.

Prepulse inactivation curves were constructed by plotting the peak current after subtraction versus the second prepulse voltage (Fig. 1C). The Boltzman relation $I/I_{max} = 1/(1 + \exp((V_{0.5} - V_m)/S))$ was fitted to data obtained in calcium bath solution (open circles) with $V_{0.5} = -93.1 \pm 0.35$ mV and $S = -7.1 \pm 0.31$ mV (n = 12), and with $V_{0.5} = -81.1 \pm 0.54$ mV and S = −6.6 ± 0.48 mV (n = 31) when calcium was replaced by manganese and 1 mM TEA, 0.2 mM d-tubocurarine and 200 nM TTX was added (filled circles). A similar shift in the inactivation characteristics caused by divalent cations has been previously described in other neuronal and

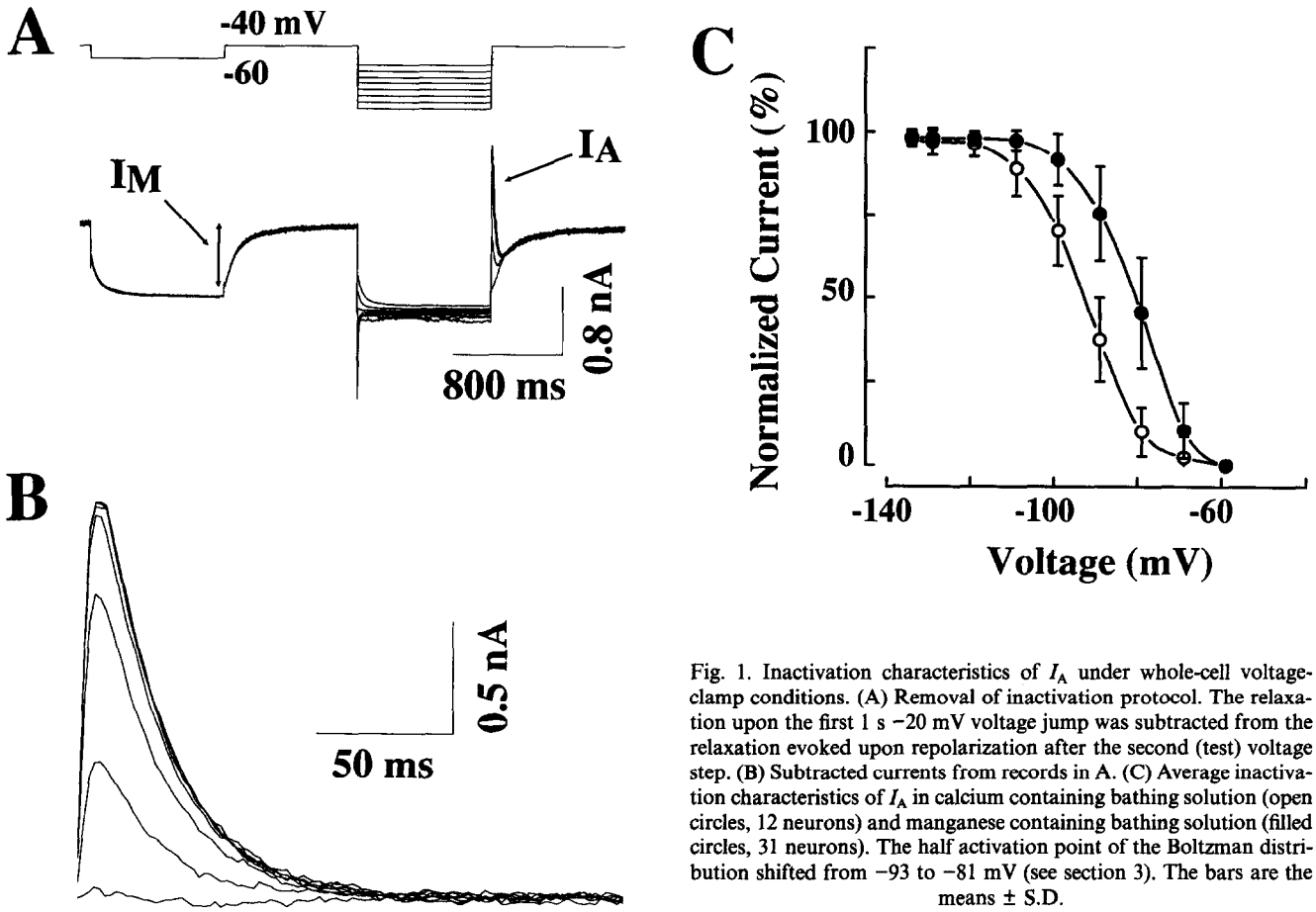

Fig. 1. Inactivation characteristics of I_A under whole-cell voltage-clamp conditions. (A) Removal of inactivation protocol. The relaxation upon the first 1 s −20 mV voltage jump was subtracted from the relaxation evoked upon repolarization after the second (test) voltage step. (B) Subtracted currents from records in A. (C) Average inactivation characteristics of I_A in calcium containing bathing solution (open circles, 12 neurons) and manganese containing bathing solution (filled circles, 31 neurons). The half activation point of the Boltzman distribution shifted from −93 to −81 mV (see section 3). The bars are the means ± S.D.

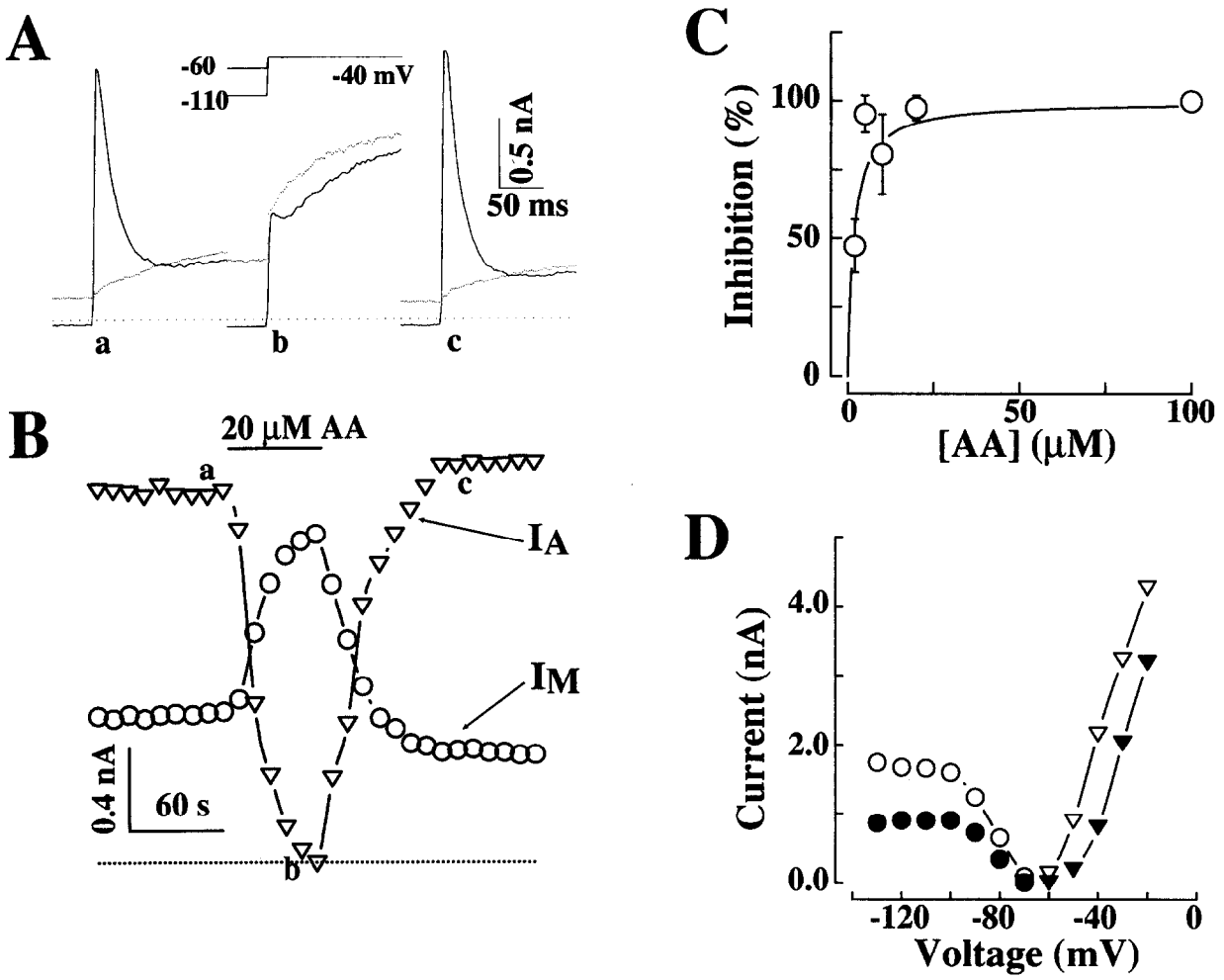

Fig. 2. Arachidonic acid suppress I_A and enhances I_M. (A) I_M relaxation evoked at −40 mV by a 1 s pre-pulse to −60 mV (dotted lines), and I_A revealed by a 1 s pre-pulse to −110 mV (which removes I_A inactivation) in control (a), in the presence of 20 μM AA (b), and after AA removal (c). Horizontal dotted line indicates zero current level. Bovine serum albumin (1.0 mg/ml) was used to remove AA from the bath. A suction pipette was positioned 150 μm from the cell to exchange the solutions. This set up was important to reveal the effect of AA at low concentrations [25]. (B) Time course of I_A inhibition (triangles) and I_M enhancement by AA in the same cell as in A. I_M was estimated as the steady-state current at −40 mV, and I_A as the peak current evoked after a 1 s pre-pulse to −110 mV, subtracted from the current evoked after a 1 s pre-pulse to −60 mV to reduce the contribution of I_M and leak. This protocol causes an underestimation of the size of I_A. (C) Concentration dependent relation for AA-mediated I_A inhibition. The hyperbolic relation Max·[AA]/([AA] + IC_{50}) was fitted to the data (solid line), with Max = 100.0 ± 4.31%, and IC_{50} = 1.75 ± 0.42 μM. The circles represent the average (from lower to higher [AA]) of 7, 3, 11, 12 and 2 independent experiments. Vertical bars are standard deviations of the mean. (D) Activation (triangles) and inactivation removal (circles) of I_A in control (open symbols) and in the presence of 10 μM AA (filled symbols). Activation values were measured at the different potentials, stepping from a holding potential of −105 mV. Inactivation removal was measured at −40 mV as the peak current evoked after 1 s prepulses to the different potentials, and after subtracting the current evoked by a 1 s pre-pulse to −60 mV, to reduce the contribution of I_M.

cardiac A-currents [27,28]. All the experiments presented below were performed in manganese extracellular solution.

Fig. 2A (continuous trace) shows the transient potassium A-current evoked by a 1 s prepulse to −110 mV. After subtracting the M-current relaxation, the mean peak current was 1,532 ± 854 pA (n = 32) (mean ± S.D.). The M-current relaxations were observed practically in isolation when the prepulse potential was −60 mV (Fig. 2A, dotted trace). Addition of arachidonic acid (AA) to the bath caused the suppression of I_A and the augmentation of I_M (Fig. 2A, center). The effect of AA on I_M has been described elsewhere [25,29–31].

After AA was removed from the bath, I_A and I_M returned to levels comparable to control (Fig. 2A, left). Fig. 2B shows that both I_A suppression (triangles) and I_M increase (circles) followed a parallel time-course. The reduction of I_A by AA was dose-dependent (Fig. 2C). The concentration estimated to cause 50% reduction was 1.75 μM (see legend of Fig. 2C). The reduction of I_A was not due to a shift of the voltage-dependency of inactivation removal (Fig. 2C, circles). The effect on the activation curve was also consistent with a lack of voltage shift (Fig. 2C, triangles). The protocol used to isolate I_A was not adequate to study the kinetics of I_A, due to imperfect subtraction of the contribution of I_M and

contamination by other currents. However, major changes in the inactivation kinetics were not observed.

Arachidonic acid can be metabolized mainly through the cyclooxygenase and the lipoxygenase pathways [6]. Neurons were preincubated in the presence of 10 μM indomethacin, a cyclooxygenase blocker. The addition of 20 μM AA in the presence of this drug caused suppression of I_A and increase of I_M. I_A was reduced by 83.0 ± 9.7%, and I_M increased by 38.0 ± 26.7% (n = 6). The role of the lipoxygenase pathway was investigated next. The M-current provides a convenient internal control, because it has been shown that blocking the lipoxygenase pathway causes I_M reduction, and that AA-mediated I_M enhancement can be prevented by lipoxygenase inhibitors [31]. In the presence of 10 μM nordihydroguaiaretic acid, a lipoxygenase inhibitor that almost suppressed I_M completely at this concentration, 5 μM AA did not increase the residual I_M, but reduced I_A by 79.9 ± 7.2% (n = 3).

10 μM eicosatetraynoic acid (ETYA), an AA analog with triple instead of double bonds that is not metabolized by the lipoxygenase or the cyclooxygenase pathways [6], suppressed I_A (Fig. 3C). I_A was reduced by 98.6 ± 2.1% (n = 6), and I_M was reduced by 56.9 ± 16.8% (n = 6). The reduction of I_M was expected because ETYA inhibits the lipoxygenase pathway [6]. Taken together, these results suggest that AA does not need to be metabolized to exert its effect on I_A.

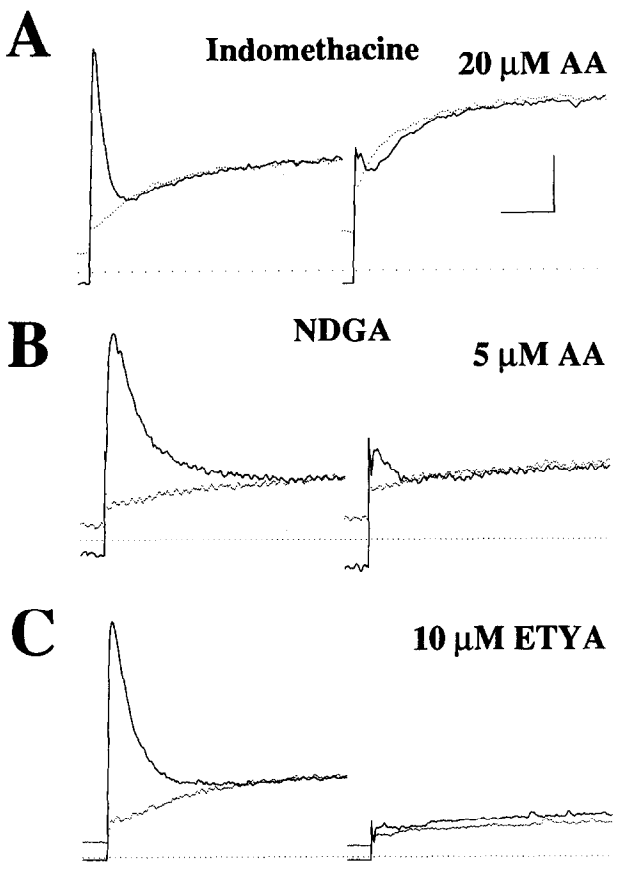

Fig. 3. Pharmacology of I_A modulation. (A) 10 μM indomethacin (a cyclooxygenase inhibitor) did not prevent I_A blockade or I_M enhancement by AA. Left, current evoked with the same protocol as in Fig. 2 in the presence of indomethacin. Right, effect of 20 μM AA in the presence of indomethacin. Horizontal scale bar is 100 ms, and vertical bar is 0.17 nA. Horizontal dotted line indicates zero current level. (B) 10 μM NDGA (a lipoxygenase inhibitor) prevented I_M enhancement by AA, but not I_A suppression. Left, control. Right, after addition of AA. Vertical bar in A correspond to 0.125 nA. (C) 10 μM ETYA (an AA analog) reduced I_M and suppressed I_A. Left, control. Right, in the presence of ETYA. Vertical scale bar in A correspond to 0.8 nA.

4. DISCUSSION

Modulation of A-currents can be classified in two groups: (a) modulation associated with shifts in the voltage-dependency, and (b) reduction or increase of the current with no apparent change in the voltage-dependency. The first group includes the action of divalent cations in neuronal [27], and this paper and cardiac cells [28], and the modulation by muscarinic [15,17] and by $GABA_B$ agonists [18] in brain neurons. The second group includes the reduction induced by α1-adrenergic agonists in dorsal raphe neurons [14], atrial [22] and ventricular [19], but see [20] myocytes, and the effect of arachidonic acid presented in this paper.

The AA concentration that caused 50% reduction of I_A (1.8 μM) is comparable to the concentration (2.8 μM) that cause 50% of the maximal I_M enhancement in these neurons [25]. These concentrations are on the same order of magnitude than the K_M of several enzymes involved in the AA cascade [6], and positions AA as a plausible second messenger mediating I_A inhibition. The possibility that AA may be involved in I_A inhibition by α1 adrenergic agonists in atrial myocytes [22] is very attractive, because there is indirect evidence that adrenergic receptor activation causes production of AA in atrium [32].

AA metabolism may not be needed to inhibit I_A, because the effect was not prevented by the cyclooxygenase inhibitor indomethacin or the lipoxygenase inhibitor NDGA. Conversely, the non-metabolizable analog ETYA, which can substitute for AA in the direct activation of potassium channels in smooth muscle cells [33], mimicked the response. Preliminary experiments suggest that mediation of AA action by protein kinase C (PKC) is unlikely, because maximal concentrations of phorbol dibutyrate that activates PKC and reduces I_M, reduced I_A very little or not at all. Further experiments are necessary to establish the mechanism of action of AA on I_A. Intracellular calcium, which is known to reduce I_A [34], and free oxygen radicals [12] are potential mediators for AA action. However, the participation of free radicals is unlikely, because the experiments were performed in the presence of manganese, which has free radical scavenging properties [35]. Although the purity of AA used in this study was greater than 99%,

the contribution of peroxides to I_A suppression cannot be ruled out.

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REFERENCES

[1] Levitan, I.B. (1988) Ann. Rev. Neurosci. 11, 119–136.
[2] Kolb, H.A. (1990) Rev. Physiol. Biochem. Pharmacol. 115, 51–91.
[3] Ordway, R.W., Singer, J.J. and Walsh, J.V. (1991) Trends Neurosci. 14, 96–100.
[4] Belardetti, F. and Siegelbaum, S.A. (1988) Trends Neurosci. 11, 232–238.
[5] Shimizu, T. and Wolfe, L.S. (1990) J. Neurochem. 55, 1–15.
[6] Needleman, P., Turk, J., Jakschik, B.A., Morrison, A.R. and Lefkowith, J.B. (1986) Ann. Rev. Biochem. 55, 69–102.
[7] Axelrod, J.A., Burch, R.M. and Jelsema, C.L. (1988) Trends Neurosci. 11, 117–123.
[8] Loffelholz, K. (1989) Biochem. Pharm. 38, 1543–1549.
[9] Piomelli, D., Volterra, A., Dale, N., Siegelbaum, S.A., Kandel, E.R., Schwartz, J.H. and Belardetti, F. (1987) Nature 328, 38–43.
[10] Kurachi, Y., Ito, H., Sugimoto, T., Shimizu, T., Miki, I. and Ui, M. (1989) Nature 337, 555–557.
[11] Piomelli, D. and Greengard, P. (1990) Trends Pharmacol. Sci. 11, 367–373.
[12] Keyser, D.O. and Alger, B.E. (1990) Neuron 5, 545–553.
[13] Rogawski, M.A. (1985) Trends Neurosci. 5, 214–219.
[14] Aghajanian, G.K. (1985) Nature 315, 501–503.
[15] Nakajima, Y., Nakajima, S., Leonard, R.J. and Yamaguchi, K. (1986) Proc. Natl. Acad. Sci. USA 83, 3022–3026.
[16] Nakayama, T. and Fozzard, H.A. (1988) Circ. Res. 62, 162–172.
[17] Akins, P.T., Surmeier, D.J. and Kitai, S.T. (1990) Nature 344, 240–242.
[18] Saint, D.A., Thomas, T. and Gage, P.W. (1990) Neurosci. Lett. 118, 9–13.
[19] Apkon, M. and Nerbonne, J.M. (1988) Proc. Natl. Acad. Sci. USA 85, 8756–8760.
[20] Tohse, N., Nakayama, H., Hattori, Y., Endou, M. and Kanno, M. (1990) Pflügers Arch. 415, 575–581.
[21] Ravens, U., Wang, X.-L. and Wetter, E. (1989) J. Pharm. Exp. Ther. 250, 364–370.
[22] Fedida, D., Shimoni, Y. and Giles, W.R. (1990) J. Physiol. 423, 257–277.
[23] Braun, A.P., Fedida, D., Clark, R.B. and Giles, W.R. (1990) J. Physiol. 431, 689–712.
[24] Doerner, D., Pitler, T.A. and Alger, B.E. (1988) J. Neurosci. 8, 4069–4076.
[25] Villarroel, A. (1993) Submitted.
[26] Adams, P.R., Brown, D.A. and Constanti, A. (1982) J. Physiol. 330, 537–572.
[27] Mayer, M.L. and Sugiyama, K. (1988) J. Physiol. 396, 417–433.
[28] Agus, Z.S., Dukes, I.D. and Morad, M. (1991) Am. J. Physiol. 261, C310–C318.
[29] Schweitzer, P., Madamba, S. and Siggins, G.R. (1990) Nature 346, 464–467.
[30] Béhé, P., Sandmeier, K. and Meves, H. (1992) Pflügers Arch. 422, 120–128.
[31] Yu, S.P., Adams, P.R. and Rosen, A.D. (1991) Soc. Neurosci. Abstr. 17, 67 (Abstract).
[32] Kurachi, Y., Ito, H., Sugimoto, T., Shimizu, T., Miki, I. and Ui, M. (1989) Pflügers Arch. 414, 102–104.
[33] Ordway, R.W., Walsh, J.V. and Singer, J.J. (1989) Science 244, 1176–1179.
[34] Chen, Q.X. and Wong, R.K.S. (1991) J. Neurosci. 11, 337–343.
[35] Kono, Y., Takahashi, M.A. and Asada, K. (1976) Arch. Biochem. Biophys. 174, 454–462.