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The Neuroprotective Agent Riluzole Activates the Two P Domain K^+ Channels TREK-1 and TRAAK

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ABSTRACT

Riluzole (RP 54274) is a potent neuroprotective agent with anticonvulsant, sedative, and anti-ischemic properties. It is currently used in the treatment of amyotrophic lateral sclerosis. This article reports that riluzole is an activator of TREK-1 and TRAAK, two important members of a new structural family of mammalian background K^+ channels with four transmembrane domains and two pore regions. Whereas riluzole activation of TRAAK is sustained, activation of TREK-1 is transient and is followed by an inhibition. The inhibitory process is attributable to an increase of the intracellular cAMP concentration by riluzole that produces a protein kinase A-dependent inhibition of TREK-1. Mutants of TREK-1 lacking the Ser residue where the kinase A phosphorylation takes place are activated in a sustained manner by riluzole. TRAAK is permanently activated by riluzole because, unlike TREK-1, it lacks the negative regulation by cAMP.

K^+ channels are widely distributed membrane proteins that are involved in diverse cellular functions (Hille, 1992) and present a large variety of properties (conductance, activation and inactivation kinetics, regulation, pharmacology and molecular structure).

A new structural family of K^+ channels has been cloned recently from mammals (Lesage and Lazdunski, 1999). These channels contain four transmembrane (TM) domains and two P domains in the same protein subunit instead of one for the voltage-gated and inward-rectifier K^+ channels. The properties, as well as the wide tissue distribution of these two P domain K^+ channels, suggest that they are background channels involved in the generation and modulation of the resting membrane potential in various cell types (Fink et al., 1996, 1998; Lesage et al., 1996; Duprat et al., 1997; Reyes et al., 1998; Lesage and Lazdunski, 1999). A particularly interesting subclass of two P domain K^+ channels is represented by TREK-1 and TRAAK, which are K^+ channels that are activated both by arachidonic acid and by membrane stretch (Patel et al., 1998; Maingret et al., 1999). In addition, TREK-1 activity is also modulated by cAMP (inhibition) and by variations of internal pH (activation at acidic PH_i) (Patel et al., 1998; Maingret et al., 1999).

Riluzole (RP 54274) is a neuroprotective agent with anticonvulsant, sedative and anti-ischemic properties (Malgouris et al., 1989; Romettino et al., 1991; Stutzmann et al., 1991; Pratt et al., 1992; Bryson et al., 1996). It is particularly effective in preventing spinal cord injury, a devastating complication of thoracoabdominal aortic surgery (Lang-Lazdunski et al., 1999). It is also very effective in retinal ischemia in models of glaucoma (Ettaiche et al., 1999). It is currently used in the treatment of amyotrophic lateral sclerosis (Bensimon et al., 1994). It has anesthetic properties at high concentrations (Mantz et al., 1992; Maclver et al., 1996). An important part of the mechanism of riluzole is probably linked to the fact that it inhibits synaptic glutamate release. This inhibition could be partly attributable to the riluzole-induced inhibition of voltage-dependent sodium channels (Doble, 1996). However, another mechanism of inhibition of glutamate release is the possible activation of background K^+ channels (Fink et al., 1998), which would lead to robust polarization, possibly both at presynaptic and at postsynaptic levels. This article reports that riluzole activates TREK-1 and TRAAK. It analyzes the mechanistic reasons that result in a sustained activation of TRAAK by riluzole whereas TREK-1 is first transiently activated and then inhibited.

Materials and Methods

Molecular Biology. Reports on the cloning of TREK-1 and TRAAK and the construction of pIRES-CD8-TREK-1 and pIRES-CD8-TRAAK have been published previously (Fink et al., 1996, 1998). We have also previously reported the construction of the protein kinase A (PKA) mutants (Patel et al., 1998).

Patch-Clamp Recordings in Transfected COS Cells. COS cells were seeded at a density of 20,000 cells per 35-mm dish 24 h

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ABBREVIATIONS: PDE, phosphodiesterase; PKA, protein kinase A; 8CPT, 8-(4-chlorophenylthio); IBMX, isobutyl methyl xanthine; DNP, dinitrophenol; MCD, mast cell degranulating.

before transfection. Cells were then transiently transfected by the classic DEAE-dextran method with 1 μg of TREK-1, TREK-1 C1, and TRAAK or 0.1 μg of PKA I and PKA II plasmids per 35-mm dish. Transiently transfected cells were visualized 48 h after transfection using the anti-CD8 antibody coated beads method.

For whole-cell and excised-patch recordings, the internal solution contained 150 mM KCl, 3 mM $MgCl_2$, 5 mM EGTA, and 10 mM HEPES at pH 7.2 with KOH, and the external solution 150 mM NaCl, 5 mM KCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES at pH 7.4 with NaOH. Cells were superfused continuously with a microperfusion system during the experiments (0.1 ml/min) performed at room temperature. A RK400 patch-clamp amplifier (Bio-Logic, Grenoble, France) was used for whole-cell as well as single-channel recordings. Data were analyzed using pClamp software (Axon Instruments, Foster City, CA).

Stock solutions of riluzole (0.1 M) were made in dimethylsulfoxide; the final solutions were made regularly before use.

The variability of the results are expressed as the standard error of the mean (S.E.M.) with n indicating the number of cells tested contributing to the mean. Statistical significance was assayed using Student's t test with a confidence level of 0.05, 0.01, or 0.001 indicated with *, **, or ***, respectively.

cAMP Radioimmunoassay. COS cells were maintained in DMEM (Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum. Twenty-four to 48 h before the assay, cells were dissociated and 2.10^5 cells were plated per 35-mm dish. When the half-confluency was reached, the cells were briefly washed, then preincubated in a solution without drugs. After 10 min, this solution was removed and replaced by an equivalent solution containing the drug. For the washing, preincubation and incubation steps, the solution used was the external medium for electrophysiology. cAMP was extracted by adding 1 ml of 65% ethanol (−20°C) on the cells immediately after removal of the incubation solution. The plates were placed at −20°C. After 3 h, the ethanol solution was collected and briefly centrifuged to eliminate cell fragments, then speed-vacuumed dried. Pellets were reconstituted with 200 μl of assay solution, and the quantification of cAMP was performed by using a commercial radioimmunoassay kit (Immunotech, Luminy, France).

Phosphodiesterase (PDE) Assays. The PDE assays were performed using the procedures described by Nicholson et al. (1989) for bovine brain PDE I, Torphy et al. (1992) for human differentiated U-937 cell PDE II, Weishaar et al. (1986) for human platelet PDE III, and Torphy et al. (1992) for human U-937 cell PDE IV. The reactions were conducted at 30°C for 30 min using [^{3}H]cAMP as substrate (0.1 μM for PDE III and 1 μM for PDE I, II, and IV). 5′-[^{3}H]AMP was recovered, then the radioactivity was measured by scintillation. For each assay, the effect of riluzole was tested at 100 μM in duplicate. In each experiment, an inhibitory compound of reference was tested simultaneously as control (8-methoxy-IBMX for PDEI, *erythro*-9–3-(2-hydroxynonyl) adenine for PDEII, milrinone for PDE III, and rolipram for PDE IV).

Results

Stimulation of TRAAK Current by Riluzole. Figure 1A shows that riluzole elicits a very significant, rapid, stable and reversible stimulation of whole-cell TRAAK current expressed in transfected COS cells, recorded at 0 mV. The TRAAK activation is dose-dependent (Fig. 1, A and B). Activation kinetics of the riluzole-activated TRAAK current are instantaneous as for the control, and no inactivation is observed (Fig. 1C). The current-potential relationship shows that riluzole activation of the TRAAK current is observed at all potentials (Fig. 1D). Activation of TRAAK currents is maintained as long as riluzole application is maintained.

The Dual Effect of Riluzole on TREK-1 Current. Riluzole has also been tested on TREK-1. Figure 2 shows that riluzole produces a dual effect on this channel. The first step of the drug action is a rapid stimulation. However, and contrary to TRAAK, this stimulation is followed by a decline of the activation and then a strong inhibition of the K^+ current (Fig. 2A). The time constant of this inhibitory effect is τ = 1.48 ± 0.44 min (n = 10). Figure 2B provides an histogram of the stimulation seen at 30 s, followed by the decrease of activity seen at 90 s. The kinetics and voltage dependencies of the TREK-1 currents obtained in the presence of riluzole at 30 s and 90 s are presented in Fig. 2, C and D.

Action of Riluzole on TREK-1 Currents Using the Excised-Patch Configuration. Figure 2E shows that ri-

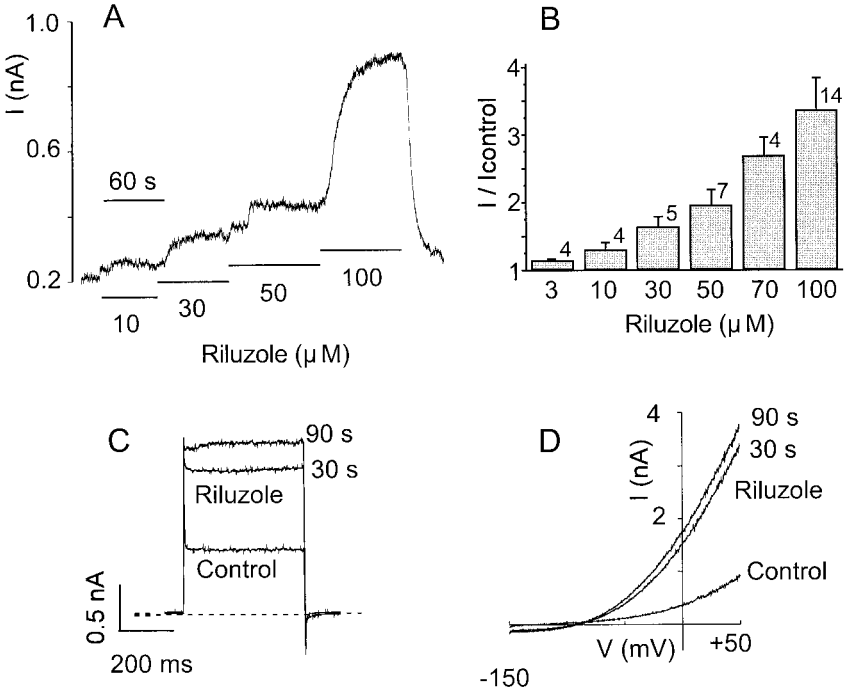

Fig. 1. Stimulation of TRAAK current by riluzole. A, example of stable stimulation of TRAAK current, recorded at 0 mV, by increasing concentrations of riluzole. B, dose-effect data of riluzole on TRAAK channel activation. C, currents elicited by voltage pulses at +50 mV, during 400 ms, before perfusion with riluzole (100 μM) (control) and after 30 s and 90 s of perfusion (n = 12, P = 2.03 10^{-4} and 1.29 10^{-5}, respectively). D, current-voltage relationships recorded with a voltage ramp protocol, 1 s in duration, from −150 mV to +50 mV under the same conditions as in C. In all cases, the holding potential is −80 mV (K^+ equilibrium potential).

luzole stimulates TREK-1 unitary channel activities in excised outside-out patches. This channel reaches a steady maximum activity level during maintained drug applications (2 min) without noticeable inactivation (data not shown). These events were not recorded in untransfected cells ($n > 10$) or mock-transfected cells ($n = 5$). Riluzole already has been found to increase TRAAK single-channel activities (Fink et al., 1998) without inactivation. These results indicate a direct action of riluzole both on TREK-1 and on TRAAK channels.

The Dual Effect of Riluzole on the TREK-1 Channel Is Associated with an Increase of Intracellular cAMP. TREK-1, unlike TRAAK, is highly sensitive to variations of intracellular cAMP and is inhibited via the PKA pathway (Fink et al., 1996; Patel et al., 1998). Figure 3A shows an inhibition of the TREK-1 current by exposure to 8-(4-chlorophenylthio) (8CPT)-cAMP (500 μM). After this inhibition, the channel cannot be reopened by 100 μM riluzole. Conversely TRAAK, which is not sensitive to cAMP treatment (Fig. 3B) (Fink et al., 1996) remains very sensitive to riluzole in the presence of 8CPT-cAMP showing that, in this case, variations of intracellular cAMP have no effect on riluzole activation.

These results suggest that the inhibitory effect of riluzole that follows TREK-1 activation is associated with a secondary effect of the drug on the PKA pathway. This hypothesis was tested with two TREK-1 constructions with point mutations that convert the two serine residues Ser334 (PK I) and Ser352 (PK II), which are potential sites for PKA phosphorylation in TREK-1 into alanines (Patel et al., 1998). Mutations at the PK I site are known to remove the sensitivity of TREK-1 to cAMP. This TREK-1 mutant can be activated by riluzole even in the presence of cAMP (Fig. 3C). The stimulatory effect of the drug is then stable, with no inhibition after the activation (Fig. 3, D and E). This result emphasizes the importance of Ser334 in the TREK-1 inhibition induced by riluzole. We have shown previously that mutations at Ser352 (PK II) are without effect on the negative regulation of the TREK-1 channel by cAMP (Patel et al., 1998). Therefore, as expected, and as described previously in this report for the wild-type TREK-1 channel, we observed a dual effect of riluzole, i.e., an activation followed by an inhibition this PK II mutant (Fig. 3, F–H).

Riluzole Increases Intracellular cAMP Levels. All these data taken together strongly suggest that riluzole produces its inhibitory effect on TREK-1 by activating the PKA pathway. This effect could be attributable to a direct stimulation of adenylate cyclase, an inhibition of PDE, or a direct stimulation of PKA. Figure 4A shows that 5 min of incuba-

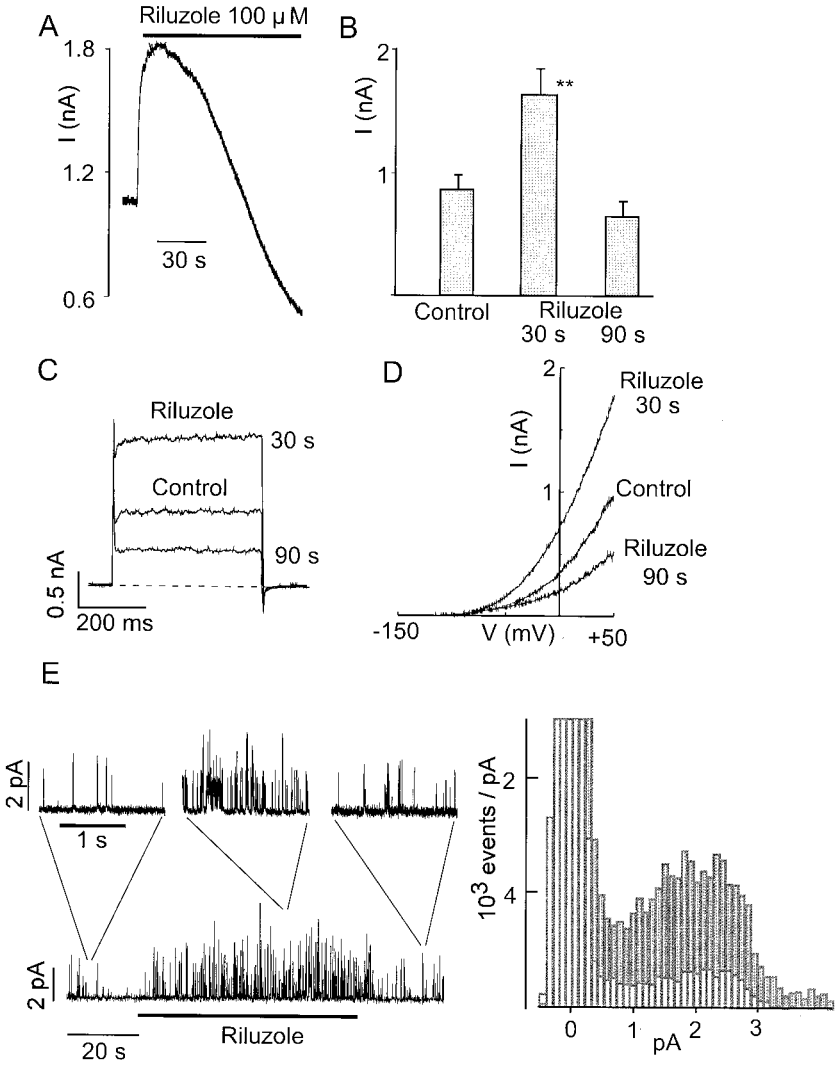

Fig. 2. Dual effect of riluzole on TREK-1 current. A, example of transient stimulation of TREK-1 current followed by an inhibition, recorded at 0 mV, during 90 s perfusion with 100 μM of riluzole. B, bar graph of TREK-1 mean currents, recorded at +50 mV, before perfusion with riluzole (100 μM) (control) and after 30 s and 90 s of perfusion ($n = 29$, $P = .002$). C, currents elicited by voltage pulses at +50 mV, during 400 ms, under the same conditions as in B. D, current-voltage relationships recorded with a voltage ramp protocol, 1 s in duration, from −150 mV to +50 mV under the same conditions as in B. In all cases, the holding potential is −80 mV (K^+ equilibrium potential). E, riluzole activates TREK-1 in outside-out patches. Reversible effects of 20 μM of riluzole on TREK-1 channel activities. The holding potential is 0 mV. Top traces, expanded traces during control, drug-induced activities and wash. (Right); superimposed amplitude histograms corresponding to control (white) and during riluzole application (gray).

tion of COS cells with 100 of μM riluzole significantly increases the intracellular level of cAMP. The effect is similar to that produced by the classic PDE inhibitor isobutyl methyl xanthine (IBMX, 1 mM). Moreover, the incubation with a mixture of riluzole and IBMX did not significantly increase the cAMP level in comparison with IBMX alone, suggesting again that the effect of riluzole is probably attributable to an inhibition of PDE (Fig. 4A). As tested in vitro, 100 μM of riluzole led to the inhibition of 36.5% of PDE I activity (assay 1: 37.1; assay 2: 35.9), 14.3% of PDE II activity (19.7 and 9), 62.1% of PDE III activity (63.1 and 61.2), and 59.6% of PDE IV (58.4 and 60.7) (data not shown).

The kinetics of the riluzole-induced cAMP increase (τ = 0.52 $\pm$ 0.03 min, n = 4) and the kinetics of the inhibition of TREK-1 current (τ = 1.48 $\pm$ 0.44 min, n = 10) are compared in Fig. 4B and are clearly within the same time range, showing the close correlation between the riluzole-induced increase of cAMP levels and the inhibition of TREK-1.

Stimulatory Effects of Riluzole on TREK-1 Currents during a Metabolic Stress. Because riluzole has potent neuroprotective effects in situations of metabolic stresses such as in brain, retinal, or spinal cord ischemia (Doble, 1996; Ettaiche et al., 1999; Lang-Lazdunski et al., 1999), we have also analyzed the effects of the drug on TREK-1 current in a situation mimicking this pathophysiologic situation using the metabolic inhibitor dinitrophenol (DNP). In the typical experiment shown in Fig. 5, B and C, a first application of riluzole in the absence of DNP led to a transient activation of TREK-1. DNP was then applied for at least a period of 10 min before a second application of riluzole. DNP, by itself, activated the channel. Because DNP is a potent metabolic inhibitor by uncoupling mitochondrial function, it decreases the intracellular ATP concentration, but also acidifies the cytoplasmic compartment. Because TREK-1 is activated at acidic values of internal pH (Maingret et al., 1999), DNP probably activates this channel by inducing an internal acidification. A second application of riluzole, in the presence of DNP, resulted into a sustained activation of TREK-1 instead of the transient activation obtained in the absence of DNP (Fig. 5A). The addition of 8CPT-cAMP led to the blockade of both the riluzole and the DNP effects. The easiest explanation is that by uncoupling oxidative phosphorylation and thereby acidifying the cytoplasm and inhibiting ATP production, DNP also inhibits the production of cAMP, which normally produces TREK-1 channel inhibition.

Discussion

Riluzole is an anticonvulsant (Romettino et al., 1991; Stutzmann et al., 1991) with anesthetic properties at high concentrations (Mantz et al., 1992; Maclver et al., 1996) and has potent neuroprotective effects both in focal and in global brain ischemia (Malgouris et al., 1989; Pratt et al., 1992; Wahl et al., 1993; Heurteaux et al., 1994) as well as in retinal and spinal cord ischemia (Ettaiche et al., 1999; Lang-Lazdunski et al., 1999). Riluzole slows down the progression of amyotrophic lateral sclerosis (Bensimon et al., 1994; Bryson et al., 1996), and the drug is now marketed for treatment of this disease. Riluzole also has protective effects in animal models of Parkinson disease (Boireau et al., 1994; Benazzouz et al., 1995; Barneoud et al., 1996) and in other models of acute neurodegenerative diseases (Mary et al., 1995). This multiple protective effect of riluzole raises the problem of its molecular site of action. Although riluzole at high concentrations partially inhibits some of the postsynaptic effect of glutamic acid (Doble, 1996), it is generally recognized that a large part of its beneficial effects is attributable to its capacity to inhibit glutamatergic transmission by blocking glutamate release (Cheramy et al., 1992; Martin et al., 1993; Doble, 1996); this effect is probably indirect. It could be

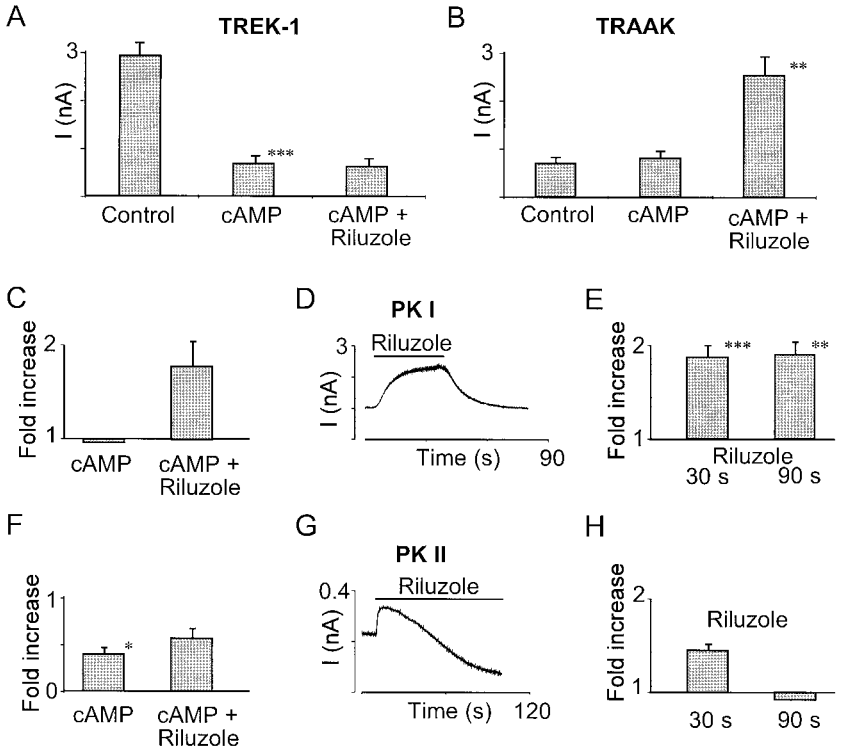

Fig. 3. Riluzole inhibits TREK-1 currents through the cAMP pathway. A, bar graph of TREK-1 mean currents, recorded at +50 mV, before perfusion with 8CPT-cAMP (500 μM) (Control), after 2 min of perfusion with cAMP alone and 90 s after adding riluzole (100 μM) to the 8CPT-cAMP perfusate (n = 5, P = 9.4 10^{-5}). B, bar graph of TRAAK mean currents, recorded at +50 mV, in the same conditions as in A (n = 6, P = .001). C, bar graph of TREK-1 PK I mutant mean currents, recorded at +50 mV, in the same conditions as in A (n = 2). D, example of stable stimulation of TREK-1 PK I current, recorded at 0 mV, during 90 s perfusion with 100 μM riluzole. E, bar graph of TREK-1 PK I mean currents, recorded at +50 mV, before perfusion with riluzole (100 μM) (Control) and after 30 s and 90 s of perfusion (n = 10, P = 6.4 10^{-4} and 1.5 10^{-3}, respectively). F, bar graph of TREK-1 PK II mutant mean currents, recorded at +50 mV, in the same conditions as in A (n = 6, P = .016). G, example of transient stimulation of TREK-1 PK II current, recorded at 0 mV, during 90 s perfusion with 100 μM riluzole. H, bar graph of TREK-1 PK II mean currents, recorded at +50 mV, before perfusion with riluzole (100 μM) (Control) and after 30 s and 90 s of perfusion (n = 10).

attributable in part to the block of voltage-dependent Na^+ channels by riluzole (Benoit and Escande, 1991), but this work shows that it also may be attributable to the action of the drug both on TREK-1 and on TRAAK potassium channels. Activation of only a small fraction of the TREK-1/TRAAK channels would be expected to have significant effects on membrane polarization and consequently pre- and postsynaptic function.

Like TWIK, TREK-1, TASK-1, and TASK-2 (Reyes et al., 1998), TRAAK is a two P domain K^+ channel with four TM domains. Like TREK-1, TRAAK is activated by arachidonic acid and is a mechanosensitive K^+ channel (Fink et al., 1998; Patel et al., 1998; Maingret et al., 1999). Unlike other K^+ channels in the same family, TRAAK is present exclusively in brain, spinal cord, and retina (Fink et al., 1998). It is abundantly expressed in synaptic terminals (Reyes et al., 2000) as well and in postsynaptic areas. It is therefore an excellent candidate to explain the protective effects of riluzole in brain ischemia as well as in spinal cord and retinal ischemia (Stutzmann et al., 1996; Springer et al., 1997; Ettaiche et al., 1999; Lang-Lazdunski et al., 1999). Riluzole also abolishes the epileptic seizures produced by the bee venom toxin mast cell degranulating (MCD) peptide (Stutzmann et al., 1991). By activating TRAAK (and possibly also TREK-1) channels, riluzole probably compensates for the inhibition of the Kv1.1, Kv1.2 and Kv1.6 voltage-dependent K^+ channels by MCD peptide. Riluzole by activating the background TRAAK channels would then have the same type of protective effects against ischemia and MCD peptide-induced convulsions as (−)cromakalim, one of the activators of ATP-sensitive K^+ channels (Quast, 1992), a drug that also prevents glutamate release (Heurteaux et al., 1993). In fact, the parallel is striking between TRAAK channels and K_{ATP} channels, because both types of channels appear to be essentially closed in normal physiologic conditions. TRAAK opens under the influence of stretch and/or of arachidonic acid (Fink et al., 1998; Maingret et al., 1999), whereas K_{ATP} channels only activate when the internal concentration of ATP decreases and the internal concentration of ADP increases (Amoroso et al., 1990).

The difference in the modes of action of riluzole on TREK-1 and TRAAK channels was surprising at first sight, because these two membrane proteins are the two closest members in the family of two P domain K^+ channels and because they are both activated by the same effectors, AA and stretch (Fink et al., 1996, 1998; Patel et al., 1998; Maingret et al., 1999). Then, why is riluzole activation of TRAAK sustained whereas activation of TREK-1 is transient and followed by an inhibition? To answer this question, we focused on the differences between TREK-1 and TRAAK properties. The most obvious difference has to do with the intracellular regulation of these two K^+ channel types. TREK-1 is inhibited by increases of cAMP that activate PKA as well as by treatments that produce an activation of PKC, whereas TRAAK is not (Fink et al., 1996, 1998). The interesting observation made in this work is that riluzole, besides directly activating both TREK-1 and TRAAK, also has the capacity of increasing intracellular cAMP concentrations. The time course of TREK-1 activation by riluzole is faster than the time course of the intracellular cAMP increase, and this is why the activation is observed before the inhibition takes place. A confirmation of this interpretation was obtained with the TREK-1 mutant in which the serine residue that is the target of cAMP-dependent phosphorylation that inhibits TREK-1 has been replaced by an alanine. This mutant has retained the activation by riluzole but has lost the subsequent inhibition.

Activation of both TREK-1 and TRAAK channels has also been observed directly in excised patches, indicating a direct interaction of the neuroprotective drug with the channel protein. Activation of the TREK-1 channel under these recording conditions is not followed by inhibition, confirming again that the inhibitory effect of riluzole is indirect and attributable to intracellular cAMP increase. Endogenous PDE activity in COS cells has been reported in different studies. However, to our knowledge no detailed molecular characterization of PDE enzymes in COS cells has been published. However, a part of the endogenous PDE activity is inhibited by rolipram, indicating that active PDE IV is expressed in these cells (Huston et al., 1996). This suggests that the increase of cAMP produced by the application of riluzole in COS cells could be attributable in part if not completely to the inhibition of endogenous PDE IV activity.

Unlike TRAAK, TREK-1 is present in many different tissues. Like TRAAK, it is present in brain, spinal cord, and retina (Fink et al., 1998; Reyes et al., 1998), but it is also present in lung, kidney, skeletal muscle, and heart. Activation of TREK-1 in the heart by riluzole could have effects on action potential duration and might induce arrhythmias. No

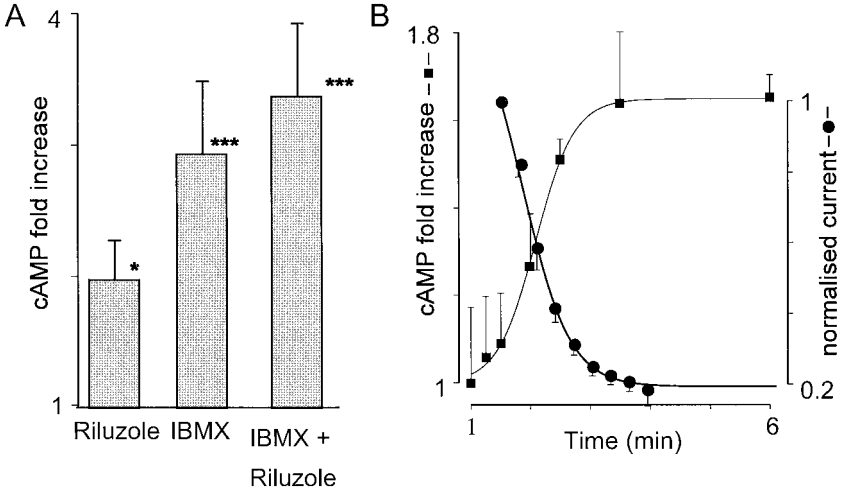

Fig. 4. Riluzole stimulates the level of cAMP in COS cells. A, bar graph of the cAMP level fold increase measured by radioimmunoassay in untransfected COS cells after 5 min perfusion with riluzole (100 μM), IBMX (1 mM), or IBMX + riluzole (n = 6, P = .039, 8.06 10^{-5} and 4.1 10^{-6}, respectively). B, comparison between the kinetics of the cAMP fold increase in untransfected COS cells (■) (τ = 0.52 ± 0.03 min, n = 4) and the inhibition of normalized TREK-1 current after perfusion with riluzole 100 μM (●) (τ = 1.48 ± 0.44 min, n = 10).

such side effects of riluzole have been reported (Wokke, 1996). The transient character of the activation of TREK-1 probably limits these side effects. In addition, by blocking voltage-sensitive Na^+ channels (Benoit and Escande, 1991), riluzole might also have type I antiarrhythmic effects.

Because riluzole is considered for potential treatments of ischemia in brain, spinal cord, and retina (Malgouris et al., 1989; Pratt et al., 1992; Wahl et al., 1993; Heurteaux et al., 1994; Stutzmann et al., 1996; Springer et al., 1997; Ettaiche et al., 1999; Lang-Lazdunski et al., 1999), it was useful to check how it could act in situations of metabolic stress assuming that neurons respond to oxidative stress similarly to the cell line used for TREK-1 expression. Our results show that chemically induced metabolic stress, which results in intracellular acidification decrease of intracellular ATP with a concomitant increase of ADP, drastically changes the action of riluzole on TREK-1 leaving the activation process and removing most of the inhibition that follows. The easiest explanation is that under these stress conditions (acidic pH and $[ATP]_{in}$ decrease), riluzole cannot increase the cAMP level high enough to inhibit the TREK-1 activity. It is confirmed by the fact that addition of 8CPT-cAMP inhibits the sustained activity of TREK-1 produced by riluzole in DNP-treated cells.

Riluzole is the first neuroprotective drug that has been administered in patients. Our results provide evidence that TRAAK and TREK-1 are probably major targets for this drug. Interestingly, riluzole at high concentrations also can act as a general anesthetic (Mantz et al., 1992), and volatile general anesthetics such as chloroform, halothane, or isoflurane have been shown to target TREK-1 channels (but they are inactive on TRAAK) (Patel et al., 1999). Results presented here might open the way for the discovery of new neuroprotective molecules acting on TREK-1 and TRAAK channels.

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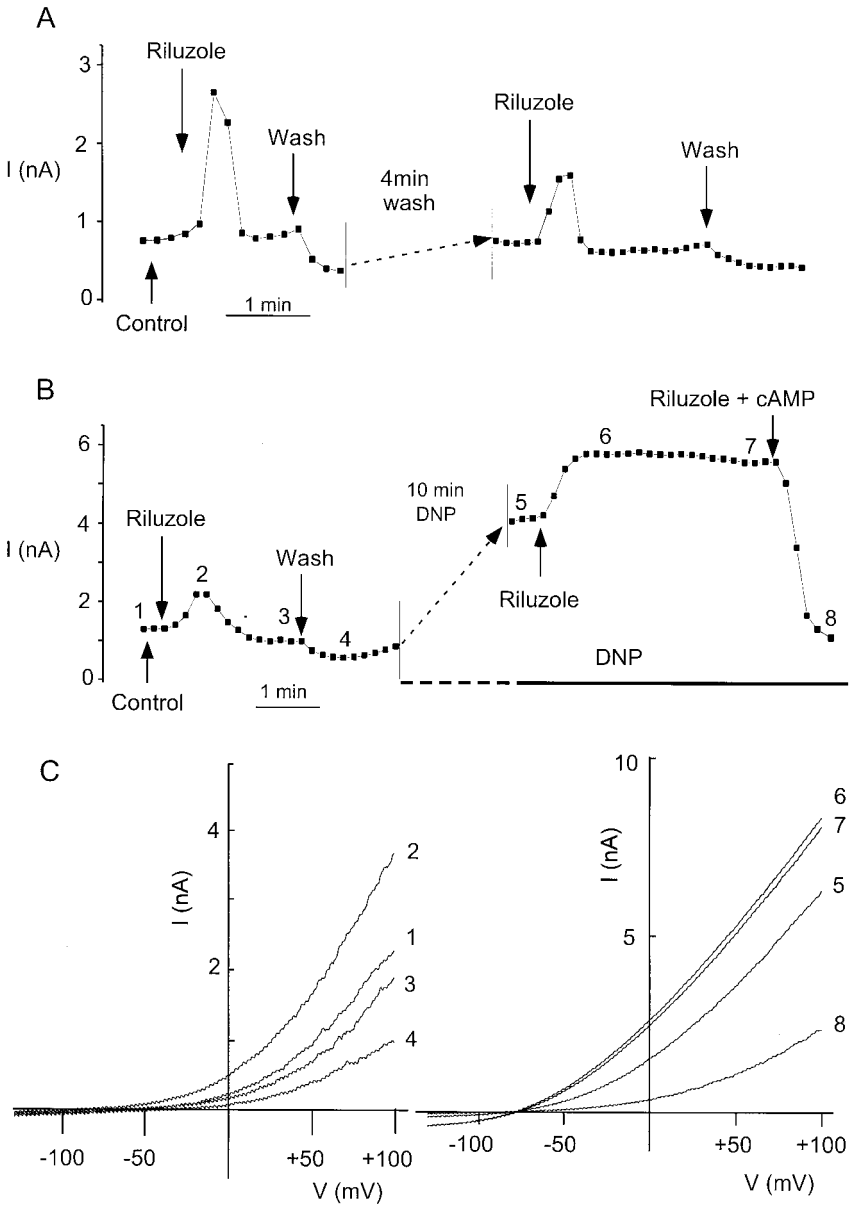

Fig. 5. Effects of riluzole on TREK-1 during a metabolic stress. The voltage-clamp protocol consisted in 1-s voltage ramps ranging from −130 mV to +100 mV applied every 10 s. A, B, monitoring of the current amplitude measured at +60 mV. A, effects of two successive applications of riluzole (100 μM) separated by a 5-min washing period. Note that the two responses are transient. B, effects of a 10-min preincubation with DNP (400 μM) on the response to riluzole. Evolution of the current under control condition (1), in the presence of riluzole (100 μM) (2, 3), during wash (4), after a 10-min application of 400 μM DNP (5) followed by the addition of riluzole (6, 7), and finally the addition of 500 μM 8CPT-cAMP (8). C, corresponding current-voltage curves of the experiment shown in B.

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