Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 9431–9435, October 1993
Neurobiology

K^+ channel openers prevent global ischemia-induced expression of c-*fos*, c-*jun*, heat shock protein, and amyloid β-protein precursor genes and neuronal death in rat hippocampus

(immediate early gene/*in situ* hybridization/hippocampus/transient forebrain ischemia)

CATHERINE HEURTEAUX, VALÉRIE BERTAINA, CATHERINE WIDMANN, AND MICHEL LAZDUNSKI*

Institut de Pharmacologie Moléculaire et Cellulaire, 660 route des Lucioles, Sophia Antipolis 06560 Valbonne, France

Communicated by Jean-Marie Lehn, June 1, 1993 (received for review March 8, 1993)

ABSTRACT **Transient global forebrain ischemia induces in rat brain a large increase of expression of the immediate early genes c-*fos* and c-*jun* and of the mRNAs for the 70-kDa heat-shock protein and for the form of the amyloid β-protein precursor including the Kunitz-type protease-inhibitor domain. At 24 hr after ischemia, this increased expression is particularly observed in regions that are vulnerable to the deleterious effects of ischemia, such as pyramidal cells of the CA1 field in the hippocampus. In an attempt to find conditions which prevent the deleterious effects of ischemia, representatives of three different classes of K^+ channel openers, (−)-cromakalim, nicorandil, and pinacidil, were administered both before ischemia and during the reperfusion period. This treatment totally blocked the ischemia-induced expression of the different genes. In addition it markedly protected neuronal cells against degeneration. The mechanism of the neuroprotective effects involves the opening of ATP-sensitive K^+ channels since glipizide, a specific blocker of that type of channel, abolished the beneficial effects of K^+ channel openers. The various classes of K^+ channel openers seem to deserve attention as potential drugs for cerebral ischemia.**

Global forebrain ischemia leads to a complete neuronal death in the CA1 field of the hippocampus after 7 days of recovery, whereas the adjacent CA3 sector and the dentate gyrus are largely more resistant (1, 2). The main factors involved in the damage of neuronal tissue following ischemia are ATP depletion (3), intracellular acidosis (4), enhanced release and/or diminished reuptake of the excitatory transmitters glutamate and aspartate (5), generation of free radicals (6), and increased Ca^{2+} influx and K^+ efflux (7, 8).

N-Methyl-D-aspartate (NMDA) antagonists and Ca^{2+} channel blockers have exhibited little ability to reduce tissue damage in animals with global ischemia (7, 9). They seem to be more helpful in focal ischemia (10, 11). Until now antagonists of α-amino-3-hydroxy-5-methylisoxazolepropionate have been considered as the best neuroprotective agents against global brain injury (12, 13). A new class of drugs has recently attracted considerable interest for protection of the ischemic heart. This is the family of ATP-sensitive K^+ channel (K_{ATP} channel) openers (KCOs). These molecules, including (−)-cromakalim, pinacidil, nicorandil, and RP 49356, are potent vasorelaxant and cardioprotective agents (for reviews see refs. 14–16). The cardioprotective effects of these drugs are completely reversed by antidiabetic sulfonylureas such as glibenclamide, which are blockers of K_{ATP} channels (17, 18).

K_{ATP} channels are present in the brain (19–21) and are particularly abundant in hippocampal structures, known to be vulnerable to the deleterious effects of ischemia. These channels are activated by the same KCOs that activate cardiac K_{ATP} channels (22). Therefore it appeared worthwhile to analyze whether the KCOs can prevent the expression of the immediate early genes c-*fos* and c-*jun* and the genes encoding the 70-kDa heat-shock protein (HSP70) and amyloid β-protein precursor (APP), which are induced following global cerebral ischemia (23–30), and to determine more directly whether KCOs are neuroprotective.

MATERIALS AND METHODS

Animals. Experiments were performed on 10- to 12-week-old, 250- to 300-g male Wistar rats (Charles River Breeding Laboratories).

Drug Treatments. (−)-Cromakalim was from Beecham Pharmaceuticals, pinacidil from Leo Pharmaceuticals, and nicorandil from Rhône-Poulenc Rorer. KCOs (10 nmol/5 μl) were administered intracerebroventricularly 30 min before the induction of cerebral ischemia and once each day during the recovery period. These doses of KCOs are similar to those previously shown to prevent seizures (31). Glipizide (1 μmol/5 μl; Pfizer Diagnostics) was injected intracerebroventricularly 20 min prior to openers. Control experiments were performed with intracerebroventricular injection of 0.9% NaCl under conditions used for openers.

Cerebral Ischemia Model. Forebrain ischemia involved occlusion of all four major extracranial arteries ("four-vessel occlusion" model) (2).

The first day, the rats were anesthesized by inhalation of 2% halothane mixed with 30% oxygen/70% nitrous oxide. Body temperature was maintained at 37°C. The vertebral arteries were irreversibly occluded by electrocoagulation. After a delay of 1 day for recovery, both common carotid arteries were clamped during 20 min in awake and spontaneously ventilating animals. Rats lost their righting reflex within 1 min of carotid clamping. Pharmacological treatments were administered 30 min prior to forebrain ischemia by an injection needle that was lowered bilaterally into the lateral ventricle. The injection needle was connected to a Hamilton syringe (10 μl) positioned in a micropump delivering the drug solution at a rate (1.25 μl/min) for 4 min. Between 1 hr and 7 days after ischemia, animals were killed by transcardial perfusion with 0.9% NaCl followed by ice-cold 1% paraformaldehyde in phosphate-buffered saline (0.15 M NaCl/0.01 M sodium phosphate, pH 7.4). The dissected brains were post-fixed in the same solution for 2 hr and then immersed overnight at 4°C in phosphate-buffered saline containing 20% sucrose. Coronal frozen sections (12 μm) at the level of the dorsal hippocampus were cut on a cryostat (Microm) at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: APP, amyloid β-protein precursor; HSP, heat-shock protein; K_{ATP} channel, ATP-sensitive K^+ channel; KCO, K_{ATP} channel opener; NMDA, *N*-methyl-D-aspartate.

*To whom reprint requests should be addressed.

−25°C, collected on 3-aminopropylethoxysilane-coated slides, and stored at −70°C until use.

***In Situ* Hybridization.** Five oligodeoxynucleotide probes were used: HSP70 (30-mer), complementary to the human sequence 5′-GTCCATGGTGCTGACCAAGATGAAGGAGAT-3′ (32); c-*jun* (60-mer), complementary to the human coding sequence 5′-CGTTGACGACGCAATCGTACTCAACCGTGGGTGACAATTGCACCAAGTACTGAAAGACAA-3′ (33); c-*fos* (45-mer), complementary to the rat sequence 5′-TTCTCGGGTTTCAACGCGGACTACGAGGCGTCATCCTCCCGCTGC-3′ (34); and two 40-mer oligonucleotides, the APP_{695} and APP_{751} probes, complementary to the mouse junctional sequences arising from two possible exon combinations: 5′-GCTGGCTGCTGTCGTGGGAACTCGGACCACCTCCTCCACG-3′ (APP_{695}) and 5′-TCTTGAGTAAACTTTGGGTTGACACGC TGC CACACACCGC-3′ (APP_{751}) (35). Oligodeoxynucleotides complementary to c-*fos*, c-*jun*, HSP70, APP_{695}, and APP_{751} probes were used as sense control probes. All probes were 3′ end-labeled with terminal deoxynucleotidyltransferase (Boehringer) and [α-[^{35}S]thio]dATP (>1000 Ci/mmol, Amersham; 1 Ci = 37 GBq) to a specific activity of 1.5×10^9 cpm/mg.

Sections were prehybridized for 1 hr at room temperature in a solution containing 4× standard saline citrate (SSC) and 1× Denhardt's solution. The slides were then rinsed 10 min in 4× SSC, acetylated for 10 min with acetic anhydride (0.5 ml/200 ml of 0.1 M triethanolamine), and dehydrated. Hybridization was carried out overnight at 42°C with c-*fos* and c-*jun* probes and at 50°C with the HSP70 oligonucleotide in 50% (vol/vol) deionized formamide/10% (wt/vol) dextran sulfate/4× SSC/1× Denhardt's solution/5% (vol/vol) sodium *N*-lauroylsarcosine/20 mM dithiothreitol/20 mM sodium phosphate containing denatured salmon sperm DNA (500 μg/ml) and yeast tRNA (250 μg/ml). For each slide, 35 ml hybridization mixture containing 3×10^5 cpm of the denatured labeled oligonucleotide was used. Slides were washed in 1× SSC/20 mM dithiothreitol at 55°C twice for 30 min before dehydration and apposition to Hyperfilm-βmax (Amersham) for 10 days.

PK 11195 Binding Procedure. The ligand [^{3}H]PK 11195 was used to label brain areas where neuronal death occurs (36). Rat brains were frozen in isopentane at −48°C. Brain sections (15 μm thick) were incubated for 60 min at 4°C in 170 mM Tris·HCl buffer (pH 7.4) containing 1 nM [^{3}H]PK 11195 (86 Ci/mmol, NEN). The nonspecific binding component was measured by incubating adjacent tissue sections in the presence of 1 mM unlabeled PK 11195. Incubation was terminated by rinsing twice for 5 min in the cold incubation buffer and twice in cold distilled water. Sections were dried, and Hyperfilm-^{3}H (Amersham) with a set of standards (Amersham microscales) was apposed for 46 days. Local tissue concentration of [^{3}H]PK 11195 was determined by quantitative densitometric analysis using a computerized image-analysis system (Alcatel TITN).

RESULTS

Levels of c-*fos* and c-*jun* mRNAs and of the transcriptional regulatory factors they encode, Fos and Jun, are highly increased in rat and gerbil brain following global and focal ischemia or traumatic brain injury (23–25).

Fig. 1 *B–F* shows the regional expression of c-*fos* and c-*jun* mRNAs in the rat hippocampus at various times of recovery following a 20-min ischemic insult. The general expression of the two probes was identical. No induction was observed in hippocampal cells of sham-control animals (Fig. 1*A*). The increase in c-*fos* and c-*jun* mRNAs within the dentate granule cells was first observed after 1 hr, was more pronounced at 6 hr, and had disappeared at 24 hr following ischemia. High c-*fos* and c-*jun* labeling was observed in CA3 and CA1 pyramidal-cell layers at 6 hr of reperfusion and persisted only in the CA1 field at 24 hr. Total labeling disappeared by 7 days postischemia.

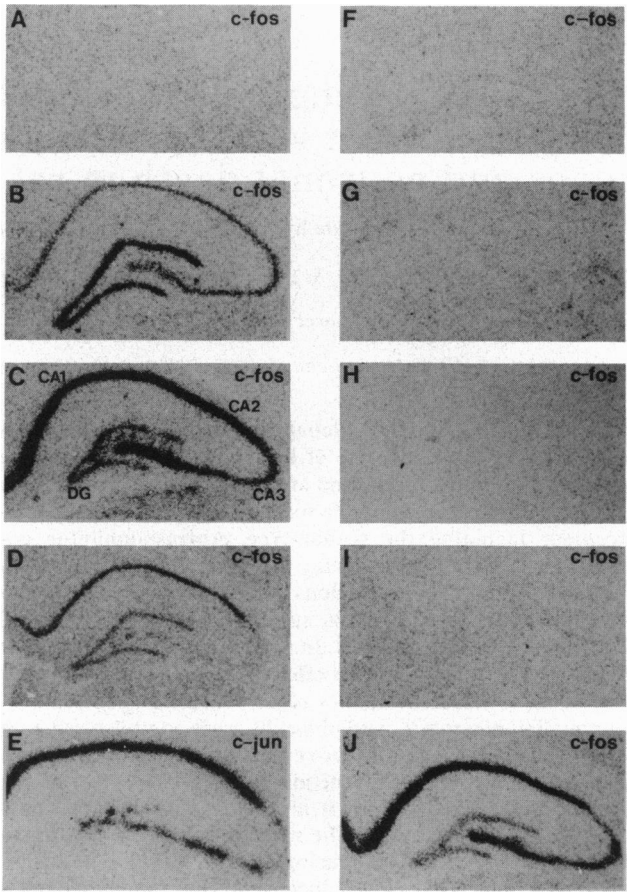

FIG. 1. Distribution of c-*fos* (*A–D* and *F–J*) and c-*jun* (*E*) mRNAs in rat hippocampus. Sections from saline-treated rats were obtained in sham control (*A*) and 1 hr (*B*), 6 hr (*C*), 24 hr (*D* and *E*), and 7 days (*F*) after a 20-min ischemia. Sections were obtained from (−)-cromakalim-pretreated rats 1 hr (*G*), 6 hr (*H*), and 24 hr (*I*) after a 20-min ischemia. Section from a rat injected with (−)-cromakalim prior to ischemia but not during 24 hr of recovery shows c-*fos* mRNA (*J*). Dark areas indicate high grain density. Structures devoid of labeling are white. Abbreviations are those used in the rat brain atlas of Paxinos and Watson (37). DG, dentate gyrus. (×10.)

Injections of (−)-cromakalim (10 nmol) before the start of cerebral ischemia and during the days of recovery totally blocked the induction of ischemia-induced expression of both c-*fos* (Fig. 1 *G–I*) and c-*jun* (data not shown). When the drug was administered just before ischemic injury but not during reperfusion, the hybridization signal appeared at 24 hr of recovery (Fig. 1*J*). Like (−)-cromakalim injections, nicorandil and pinacidil injections (10 nmol) prevented the increase of both immediate early genes following global ischemia (data not shown).

HSPs play an important role in the response to stressful conditions of various cell types (for a review see ref. 38). Induction of the synthesis of HSP70 was previously observed in ischemia (26, 27).

Fig. 2 *B–E* shows that the regional and temporal profile of HSP70 mRNA expression after transient ischemia in rat hippocampus is the same as for c-*fos* and c-*jun*. Dentate granule cells and CA3 neurons showed transient induction of HSP70 mRNA during the first 6 hr of recovery. However, at 24 hr after ischemia only CA1 pyramidal cells demonstrated a significant hybridization, which disappeared after 7 days.

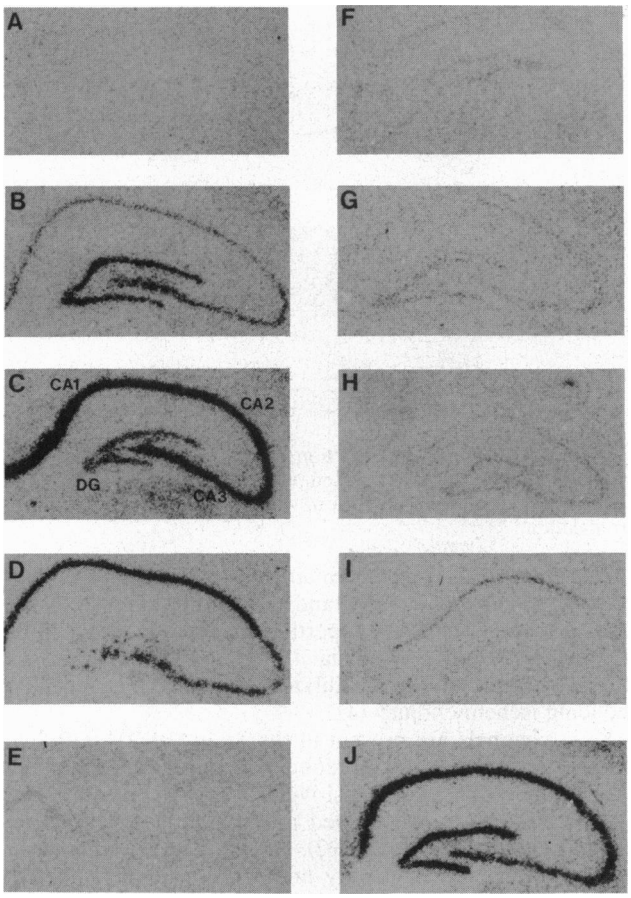

FIG. 2. Distribution of HSP70 mRNA in rat hippocampus. Sections from saline-treated rats were obtained in sham control (*A*) and 1 hr (*B*), 6 hr (*C*), 24 hr (*D*), and 7 days (*E*) after a 20-min ischemia. Sections were obtained at 1 hr (*F*), 6 hr (*G*), 24 hr (*H*), and 7 days (*I*) after ischemia in nicorandil-pretreated rats and at 24 hr after ischemia in a rat pretreated with a single nicorandil injection prior to ischemia (*J*). (×10.)

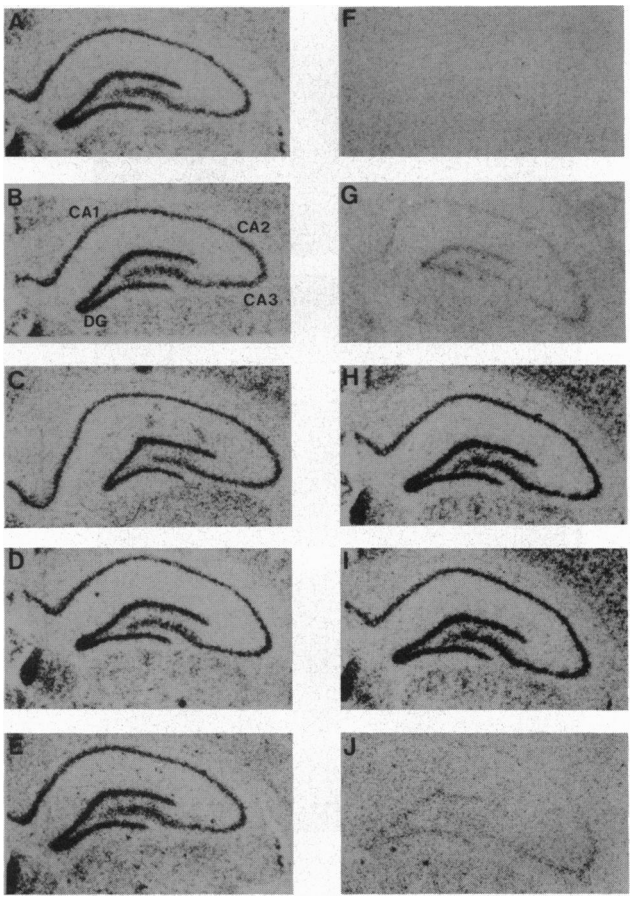

FIG. 3. Distribution of APP_{695} (*A–E*) and APP_{751} (*F–J*) mRNAs in rat hippocampus. Sections were obtained from saline-treated rats in sham control (*A* and *F*) and 1 hr (*B* and *G*), 24 hr (*C* and *H*), and 7 days (*D* and *I*) after 20 min of ischemia. Sections were obtained from a pinacidil-pretreated rat 7 days after ischemia (*E* and *J*). (×10.)

Fig. 2 *F–I* shows that pretreatment with nicorandil before ischemia and during recovery prevented the induction of HSP70 mRNA induced by the ischemic insult. However, a single injection of nicorandil, just before ischemia, blocked the increase in hippocampal HSP70 labeling observed after 1 and 6 hr of recovery but did not prevent a later induction of HSP70 mRNA in CA1 neurons at 24 hr (Fig. 2*J*).

The three isoforms of APP—APP_{695}, APP_{751}, and APP_{770}—are derived from alternative splicing of a single gene (35). Alterations of APP expression have previously been observed following ischemia (28, 29).

Fig. 3 shows the expression of APP_{695} (*B–D*) and APP_{751} (*F–H*) mRNAs in the rat hippocampus following a 20-min ischemic insult. Unlike APP_{751} mRNA, which was absent in hippocampus of sham-operated rats (Fig. 3*F*), APP_{695} mRNA was abundant in the granular layer of dentate gyrus and in pyramidal cells in layers CA1 through CA3 of control rat (Fig. 3*A*). The expression of APP_{695} mRNA did not change after global ischemia and recovery for 7 days (Fig. 3 *B–D*). Pretreatment with KCOs such as pinacidil had no effect (Fig. 3*E*). Conversely, APP_{751} mRNA, which encodes the Kunitz-type protease-inhibitor domain, was induced at 24 hr post-ischemia (Fig. 3*H*), as previously described (39), peaked at 3 days (data not shown), and remained elevated until 7 days (Fig. 3*I*). KCOs such as pinacidil blocked the induction of APP_{751} following global ischemia (Fig. 3*J*).

Fig. 4 shows more directly the neuroprotective effects of KCOs by following the appearance of peripheral-type benzodiazepine binding sites, which are associated with reactive astroglia and macrophage invasion following an ischemic insult and which provide an excellent marker of neuronal degeneration (36). After an ischemic insult of 20 min and 7 days of recovery, an increase in [^{3}H]PK 11195 labeling was particularly observed in the pyramidal-cell layer of the CA1 field and the hilus (Fig. 4 *B* and *D*). Pretreatment with (−)-cromakalim reduced the ischemia-induced increase of [^{3}H]PK 11195 binding and thus protected against ischemia-induced loss of CA1 pyramidal cells (Fig. 4 *C* and *D*). Counting of intact cells and density of neurons on histologic sections showed that delayed neuronal death was observed in 78% of CA1 neurons 7 days after ischemia and that 65% of the CA1 cells survived at 7 days of reperfusion after KCO treatment.

Since the sulfonylurea glipizide is a potent blocker of K_{ATP} channels after they have been activated by KCOs (22), we analyzed the effect of glipizide on the protection provided by (−)-cromakalim. For each probe used (c-*fos*, c-*jun*, HSP70, APP), glipizide completely antagonized the effect of KCOs on the induction of expression following ischemic injury. Fig. 5*A* shows that a hippocampal expression of HSP70 mRNA could be induced, as in the control (Fig. 2*B*), 1 hr after ischemia if glipizide had been injected prior to (−)-cromakalim (before ischemia), whereas, as previously observed, HSP70 induction was totally prevented by injection of the KCO alone (Fig. 5*B*).

DISCUSSION

KCOs are mainly known for their vasorelaxant activities and for their use as antihypertensive agents (14–16). The bene-

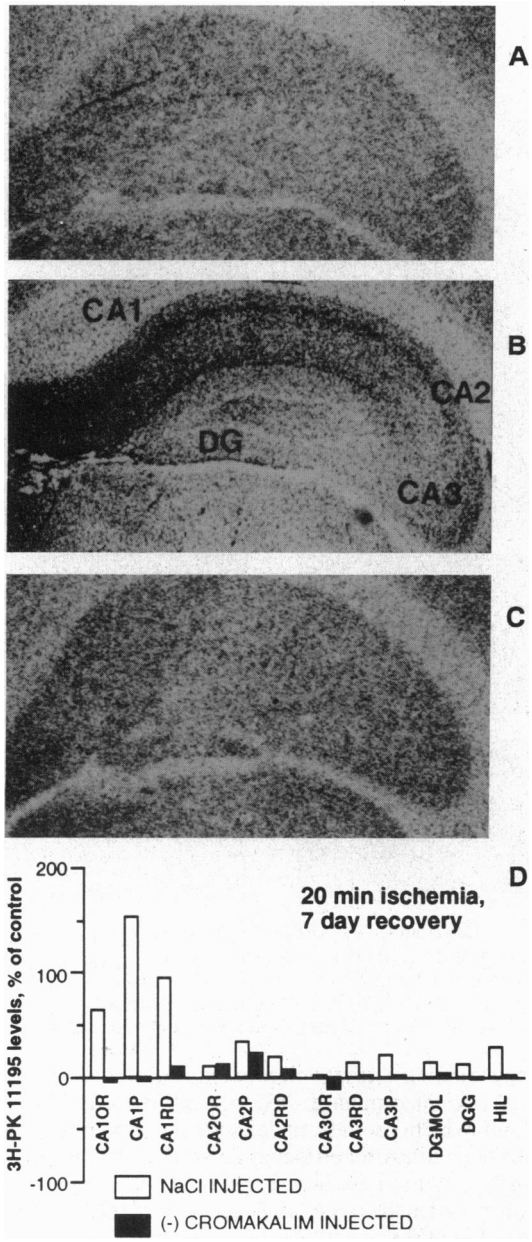

FIG. 4. Distribution of [^{3}H]PK 11195 binding in rat hippocampus. (*A–C*) Sections were obtained from saline-treated rats in sham control (*A*) and 7 days after a 20-min ischemia and from a (−)-cromakalim-pretreated rat 7 days after ischemia (*C*). (×23.) (*D*) Computerized image analysis of autoradiographs giving averages of the percentage of [^{3}H]PK 11195 binding at 7 days of recovery in (−)-cromakalim-pretreated (filled bars) and saline-pretreated (open bars) rat hippocampus. These data are means ± SD of optical density determination, calculated from 20 bilateral measurements on four sections per animal in the three rats of each experimental group. Variations were assessed with a two-tailed Student *t* test. Abbreviations are those used in ref. 37: OR, stratum oriens; P, stratum pyramidal; RD, stratum radiatum; DGMOL and DGG, molecular and granule layers of dentate gyrus; HIL, hilus of dentate girus.

ficial effects are linked to the induction of a hyperpolarization of the smooth muscle that prevents excessive Ca^{2+} entry.

(−)-Cromakalim, pinacidil, and RP 49356 have also been shown to open K_{ATP} channels in cardiac myocytes (ref. 40; reviewed in refs. 14–16). Their action is associated with a shortening of the cardiac action potential that (*i*) prevents Ca^{2+} entry that would normally occur during the plateau phase and, (*ii*) consequently, decreases the consumption of ATP which is necessary in normal conditions for the various

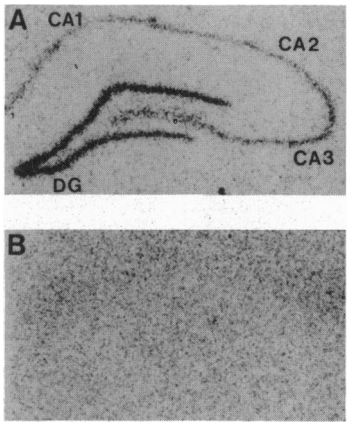

FIG. 5. HSP70 mRNA induction following global ischemia and 1 hr of recovery. (*A*) Rat injected with glipizide 20 min prior to (−)-cromakalim administration. (*B*) Rat injected with (−)-cromakalim.

Ca^{2+} pumps in charge of maintaining a low intracellular Ca^{2+} concentration. Saving ATP and preventing excessive Ca^{2+} entry are two important properties for potential drugs in the treatment of cardiac ischemia. It is therefore not surprising that (−)-cromakalim, pinacidil, and RP 52891 are capable of reducing ischemic injury (41).

K_{ATP} channels are present in the brain (19–21) with high density in substantia nigra, globus pallidus, cerebellum, and hippocampus. They are sensitive to external concentrations of glucose (42) and are opened by anoxia (20, 22, 43). They are also opened by KCOs (22). They are present in hippocampus both presynaptically and postsynaptically (44–47) and are particularly situated in mossy fibers which are associated with glutamate release (48).

Brain ischemia is associated with an excessive extracellular level of glutamate (5), probably mainly due to the fact that the high intracellular Na^+ concentration produced by ischemia prevents glutamate reuptake (after it has been liberated) through the glutamate–Na^+ cotransporter (49). On the other hand, an important extracellular glutamate concentration will produce a strong stimulation of NMDA receptors and an increased Ca^{2+} entry into target neurons that will be responsible for neuronal death (5, 7).

Two important properties can be expected from KCOs: (*i*) they should hyperpolarize synaptic terminals and thus massively prevent Ca^{2+} entry and consequently glutamate release (22, 42, 46) and (*ii*) they should hyperpolarize glutamate-sensitive neurons, thus conferring resistance to the depolarization induced by the various glutamate receptors and preventing the relief of the Mg^{2+} block of the NMDA receptor channel, which is known to be voltage-dependent and facilitated by a depolarization (50). For all these reasons KCOs should be expected to have a protective action against ischemia.

The deleterious effects of ischemia are associated with the induction of expression of mRNAs encoding the Fos and Jun nuclear proteins (23–25). Forebrain global ischemia produced an induction of these immediate early genes which at 24 hr postischemia remained intense only in the hippocampal CA1 field.

HSPs, and particularly HSP70, are synthesized in a variety of stress-related conditions, including transient ischemia (26, 27). Both temporal and spatial patterns of HSP70 mRNA induced expression following ischemia have been found in this work to be similar to expression patterns of c-*fos* and c-*jun*. The expression of c-*fos*, c-*jun*, and HSP70 mRNA was prevented by treatment with KCOs before ischemia and by treatment during reperfusion, indicating that these drugs

abolish the stress effects created by the ischemic insult. When the drug is injected only before ischemia and not during recirculation, KCOs are neuroprotective just for 1 day.

Many studies have shown the expression of APP following injuries of the central nervous system (28, 29, 51). One of the APP forms is APP_{751}, which contains a 56-amino acid domain very homologous to the Kunitz family of serine protease inhibitors (51). The other molecular form, APP_{695}, which derives from alternative splicing of the APP gene, does not contain this Kunitz inhibitor-like domain (35). The transcriptional expression of APP_{695} was not affected by ischemia, whereas the level of mRNA for APP_{751} was greatly increased 24 hr after ischemia, as also was observed by Northern blot analyses (39). This increase occurred in granule cells of the dentate gyrus and in pyramidal cells of CA1 and CA3 fields and it persisted in the CA1 region until 7 days after ischemia. Pinacidil injected before ischemia clearly blocked the expression of APP_{751}.

All these results taken together show that the different types of KCOs prevent the ischemia-induced expression of very different genes. They are in complete agreement with results involving cell counting in the CA1 sector or [^{3}H]PK 11195 as a marker of cell death (36).

One of the important properties of K_{ATP} channels is that, once they have been opened (e.g., by KCOs), they can be blocked with high potency by sulfonylureas such as glibenclamide or glipizide (17, 18, 22, 40, 42, 47, 52, 53). Indeed the beneficial effects of KCOs against the series of events produced by ischemia were suppressed when openers were administered in the presence of glipizide before ischemia was started.

In conclusion, this work shows beneficial effects of KCOs against global ischemia, as previously suggested (22). These effects are due to the action of these different compounds on K_{ATP} channels (probably at both pre- and postsynaptic levels). To find a practical application for these observations in drug therapy, it will be necessary (*i*) to synthesize KCOs which pass the blood–brain barrier and (*ii*) to develop openers with an increased specificity for brain K_{ATP} channels, to avoid effects of these compounds on blood pressure. This seems to be feasible, since K_{ATP} channels in brain and in vascular smooth muscle belong to different subtypes (54). Moreover, it is probable that KCOs will work better in hypoxic or ischemic than in normoxic conditions, since their action is known to be facilitated in the presence of ADP (52, 55).

The mechanisms by which KCOs prevent the deleterious effects of ischemia are probably similar to those which make them potentially useful compounds against various types of epileptic seizures (31, 56). Because this class of compounds prevents the expression of mRNAs related to APP, it might be interesting to investigate whether they would also be useful in preventing the development of amyloid accumulation in Alzheimer disease itself.

We thank G. Jarretou, F. Aguila, and C. Roulinat for expert technical assistance and Dr. Plotkine and his lab for useful advice on ischemia methods. This work was supported by the Centre National de la Recherche Scientifique and the Fondation pour la Recherche Médicale.

1. Ito, U., Spatz, M., Walker, J. T., Jr., & Klatzo, I. (1975) *Acta Neuropathol.* **32**, 209–223.
2. Pulsinelli, W. A. & Brierley, J. B. (1979) *Stroke* **10**, 267–272.
3. Siesjö, B. K. (1978) in *Brain Energy Metabolism*, ed. Siesjö, B. K. (Wiley, New York).
4. Siesjö, B. K. (1988) *Neurochem. Pathol.* **9**, 31–88.
5. Benveniste, H., Drejer, J., Schousboe, A. & Diemer, N. H. (1984) *J. Neurochem.* **43**, 1369–1374.
6. Kontos, H. A. & Wei, E. P. (1986) *J. Neurosurg.* **64**, 803–807.
7. Siesjö, B. K. & Bengtsson, F. (1989) *J. Cereb. Blood Flow Metab.* **9**, 127–140.
8. Nicholson, C., Bruggencate, G. T., Steinberg, R. & Stöckle, H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1287–1290.
9. Buchan, A., Hui, L. & Pulsinelli, W. A. (1991) *J. Neurosci.* **11**, 1049–1056.
10. Zivin, J. A. & Choi, D. W. (1991) *Sci. Am.* **265**, 36–43.
11. Takakura, S., Susumu, T., Satoh, H., Mori, J., Shiino, A. & Handa, J. (1991) *Jpn. J. Pharmacol.* **56**, 547–550.
12. Sheardown, M. J., Nielsen, E. O., Hansen, A. J., Jacobsen, P. & Honoré, T. (1990) *Science* **247**, 571–574.
13. Nellgard, B. & Wieloch, T. (1992) *J. Cereb. Blood Flow Metab.* **12**, 2–6.
14. Edwards, G. & Weston, A. H. (1990) *Trends Pharmacol. Sci.* **11**, 417–422.
15. Escande, D. & Cavero, I. (1992) *Trends Physiol. Sci.* **13**, 269–272.
16. Quast, U. (1992) *Fundam. Clin. Pharmacol.* **6**, 279–293.
17. Schmid-Antomarchi, H., De Weille, J. R., Fosset, M. & Lazdunski, M. (1987) *J. Biol. Chem.* **262**, 15840–15844.
18. Fosset, M., De Weille, J. R., Green, R. D., Schmid-Antomarchi, H. & Lazdunski, M. (1988) *J. Biol. Chem.* **263**, 7933–7936.
19. Ashford, M. L., Sturgess, N. C., Traut, N. J., Gardner, N. J. & Hales, C. N. (1988) *Pflügers Arch.* **412**, 297–304.
20. Mourre, C., Ben, Ari, Y., Bernardi, H., Fosset, M. & Lazdunski, M. (1989) *Brain Res.* **486**, 159–164.
21. Mourre, C., Widmann, C. & Lazdunski, M. (1990) *Brain Res.* **519**, 29–43.
22. Schmid-Antomarchi, H., Amoroso, S., Fosset, M. & Lazdunski, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3489–3492.
23. Uemura, Y., Kowall, N. W. & Beal, M. F. (1991) *Brain Res.* **542**, 343–347.
24. Wessel, T. C., Tong, H. J. & Volpe, B. T. (1991) *Brain Res.* **567**, 231–240.
25. Jorgensen, M. B., Deckert, J., Wright, D. C. & Gehlert, D. R. (1989) *Brain Res.* **484**, 393–398.
26. Nowak, T. S., Jr. (1991) *J. Cereb. Blood Flow Metab.* **11**, 432–439.
27. Kawagoe, J., Abe, K. & Kogure, K. (1992) *Brain Res.* **599**, 197–203.
28. Stephenson, D. T., Rash, K. & Clemens, J. A. (1992) *Brain Res.* **593**, 128–135.
29. Wakita, H., Tomimoto, H., Akiguchi, I., Ohnishi, K., Nakamura, S. & Kimura, J. (1992) *Neurosci. Lett.* **146**, 135–138.
30. Doucet, J. P., Squinto, S. P. & Bazan, N. G. (1990) *Mol. Neurobiol.* **4**, 27–55.
31. Gandolfo, G., Romettino, S., Gottesmann, C., Van Luijtelaar, G., Coenen, A., Bidard, J.-N. & Lazdunski, M. (1989) *Eur. J. Pharmacol.* **167**, 181–183.
32. Hunt, S. & Morimoto, R. I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6455–6459.
33. Angel, P., Allegretto, E. A., Okino, S. T., Hattori, K., Boyle, W. J., Hunter, T. & Karin, M. (1988) *Nature (London)* **332**, 166–171.
34. Curran, T., Gordon, M. B., Rubino, K. L. & Sambucetti, L. C. (1987) *Oncogene* **2**, 79–84.
35. Yamada, T., Sasaki, H., Dohura, K., Goto, I. & Sakaki, Y. (1989) *Biochem. Biophys. Res. Commun.* **158**, 906–912.
36. Benavidès, J., Capdeville, C., Dauphin, F., Dubois, A., Duverger, D., Fage, D., Gotti, B., MacKenzie, E. T. & Scatton, B. (1990) *Brain Res.* **522**, 275–289.
37. Paxinos, G. & Watson, C. (1982) *The Rat Brain in Stereotaxic Coordinates* (Academic, New York), pp. 54–56.
38. Welch, W. J. (1992) *Physiol. Rev.* **72**, 1063–1081.
39. Abe, K., Tanzi, R. E. & Kogure, K. (1991) *Neurosci. Lett.* **125**, 172–174.
40. Escande, D., Thuringer, D., Leguern, S. & Cavero, I. (1988) *Biochem. Biophys. Res. Commun.* **154**, 620–625.
41. Grover, G. J., Dzwonczyk, S. & Sleph, P. G. (1990) *Eur. J. Pharmacol.* **191**, 11–18.
42. Amoroso, S., Schmid-Antomarchi, H., Fosset, M. & Lazdunski, M. (1990) *Science* **247**, 852–854.
43. Jiang, C. & Haddad, G. G. (1989) *J. Neurophysiol.* **66**, 103–111.
44. Ben Ari, Y. & Lazdunski, M. (1989) *Eur. J. Pharmacol.* **165**, 331–332.
45. Ben Ari, Y., Krnjevic, K. & Crepel, V. (1990) *Neurosciences* **37**, 55–60.
46. Abele, A. E. & Miller, R. J. (1990) *Neurosci. Lett.* **115**, 195–200.
47. Politi, D. M. T. & Rogawski, M. A. (1991) *Mol. Pharmacol.* **40**, 308–315.
48. Mourre, C., Widmann, C. & Lazdunski, M. (1991) *Brain Res.* **540**, 340–344.
49. Nicholls, D. & Attwell, D. (1990) *Trends Pharmacol. Sci.* **11**, 462–468.
50. Ascher, P. & Nowak, L. (1987) *Trends Neurosci.* **10**, 284–288.
51. Tanzi, R. E., McClatchey, A. I., Lamperti, E. D., Gusella, J. F. & Neve, R. L. (1988) *Nature (London)* **331**, 528–530.
52. Dunne, M. J. & Petersen, O. H. (1991) *Biochim. Biophys. Acta Rev. Biomembr.* **1071**, 67–82.
53. Häusser, M., De Weille, J. R. & Lazdunski, M. (1991) *Biochem. Biophys. Res. Commun.* **174**, 909–914.
54. Miller, R. J. (1990) *Trends Neurosci.* **13**, 197–199.
55. Allard, B. & Lazdunski, M. (1992) *Pflügers Arch.* **422**, 185–192.
56. Alzheimer, C. & Ten Bruggencate, G. (1988) *Naunyn-Schmiedebergs Arch. Pharmakol.* **337**, 429–434.