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K^+ at Concentrations Reached in the Extracellular Space During Neuronal Activity Promotes a Ca^{2+}-Dependent Glycogen Hydrolysis in Mouse Cerebral Cortex

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The effect of increasing $[K^+]_o$ on ^{3}H-glycogen levels was examined in mouse cerebral cortical slices. K^+ stimulates in a time- and concentration-dependent manner the hydrolysis of ^{3}H-glycogen. Over 70% of the maximal effect is reached within 30 sec and the EC_{50} for the glycogenolytic action of K^+ is 11 mM. Significant ^{3}H-glycogen hydrolysis occurs at 5–12 mM $[K^+]_o$, concentrations reached by the ion in the extracellular space during neuronal activity. The K^+-evoked glycogenolysis is Ca^{2+}-dependent, and is inhibited by Ca^{2+}-channel blockers such as Ni^{2+} and Mn^{2+}, but not by Cd^{2+}, nifedipine, and ω-conotoxin. Furthermore, the effect of K^+ is not enhanced by the Ca^{2+}-channel agonist Bay K 8644. This type of pharmacological profile suggests that the activation of voltage-sensitive Ca^{2+} channels of the T subtype mediates the glycogenolytic action of K^+. This set of observations suggests that K^+ released in the extracellular space by active neurons may promote the mobilization of energy substrates and therefore play a role in the coupling between neuronal activity and energy metabolism.

The concentration of K^+ in the extracellular space increases during neuronal activity (Somjen, 1979; Nicholson, 1981; Sykova, 1983). Thus, from basal levels ranging between 2.5 and 3.5 mM, $[K^+]_o$ can reach 5–12 mM during electrical stimulation (Benninger et al., 1980; Gardner-Medwin and Nicholson, 1983; Sykova, 1983) or iontophoretic application of excitatory amino acids such as glutamate or aspartate (Pumain and Heinemann, 1985). This increase in $[K^+]_o$ is accompanied by a marked decrease in $[Ca^{2+}]_o$ (Benninger et al., 1980; Nicholson, 1981; Pumain and Heinemann, 1985). During pathological conditions, such as spreading depression, hypoxia, or ischemia, $[K^+]_o$ can reach even higher levels, up to 30–40 mM (Sykova, 1983, and references therein).

Glycogen is the single largest energy reserve of the nervous system (Watanabe and Passonneau, 1973). Its turnover rate is rapid (Lajtha et al., 1981) and influenced by several neurotransmitters (Siesjö, 1978). Thus we have previously described the glycogenolytic action of vasoactive intestinal peptide (Magistretti et al., 1981) and adenosine (Magistretti et al., 1986) in mouse cerebral cortical slices. Glycogen hydrolysis is also promoted by norepinephrine, serotonin, and histamine (Quach et al., 1978, 1980, 1982). Furthermore, a previous report had indicated that very high concentrations of K^+, i.e., 50 mM, could stimulate the hydrolysis of ^{3}H-glycogen newly synthesized by mouse cerebral cortical slices (Quach et al., 1978). In order to assess the functional relevance of this observation, we have examined the effect of K^+ concentrations reached in the extracellular space during neuronal activity under physiological conditions. We have observed that K^+ at concentrations ranging between 5 and 12 mM can stimulate the hydrolysis of glycogen. This effect is blocked by Ca^{2+}-channel blockers such as Ni^{2+} and Mn^{2+}, but not by Cd^{2+}, nifedipine, and ω-conotoxin, and is strongly enhanced during blockade of K^+ channels of the A subtype.

Materials and Methods

Swiss male albino mice (20–25 gm) were used throughout this study; they were maintained in an alternating 12 hr light/12 hr dark cycle and had free access to food and water.

Cerebral cortical slices were prepared as follows (Magistretti et al., 1981). Mice were decapitated and their brains rapidly removed. The cerebral cortex was then dissected on ice and immediately placed in a modified Krebs–Ringer bicarbonate buffer (KRG), pH 7.4, containing (in mM concentration) NaCl, 120; KCl, 3; $CaCl_2$, 2.6; $MgSO_4$, 0.67; KH_2PO_4, 1; glucose, 3; $NaHCO_3$, 27.5, previously gassed with O_2:CO_2 (95:5). In experiments in which the effects of Co^{2+} and Cd^{2+} on K^+-evoked glycogenolysis were tested, sulfates and phosphates in the buffer solution were replaced by chloride ions at the same molarity, i.e., $MgCl_2$ for $MgSO_4$ and KCl for KH_2PO_4 (Fig. 1*B*, Table 2). This buffer was also used in 2 control experiments with Ni^{2+} and Mn^{2+}; no difference was observed between these buffers in the effects of Ni^{2+} and Mn^{2+} (see legend to Fig. 1). The dissected cortices (usually 2 per experiment) were then placed on a McIlwain tissue chopper, their ventral aspect facing the chopping plate, and 250 μm slices were prepared. The plate was then rotated by 90° and the cortices were cut again. The slices were then resuspended in ice-cold KRG (6 ml/cortex) and incubated for 15 min at 37°C under continuous gassing (O_2:CO_2, 95:5) and vigorous shaking. After replacing the medium, 270 μl of the slice suspension were distributed into individual polypropylene test tubes, and 10 μl ^{3}H-glucose (20 nmol) were added. After gassing with a stream of O_2:CO_2 (95:5), the tubes were capped and incubated at 37°C. After 30 min, salts and/or drugs (20 μl) were added for 10 min. Blockers were added 3 min before the salts. The tubes were then centrifuged, and the supernatant

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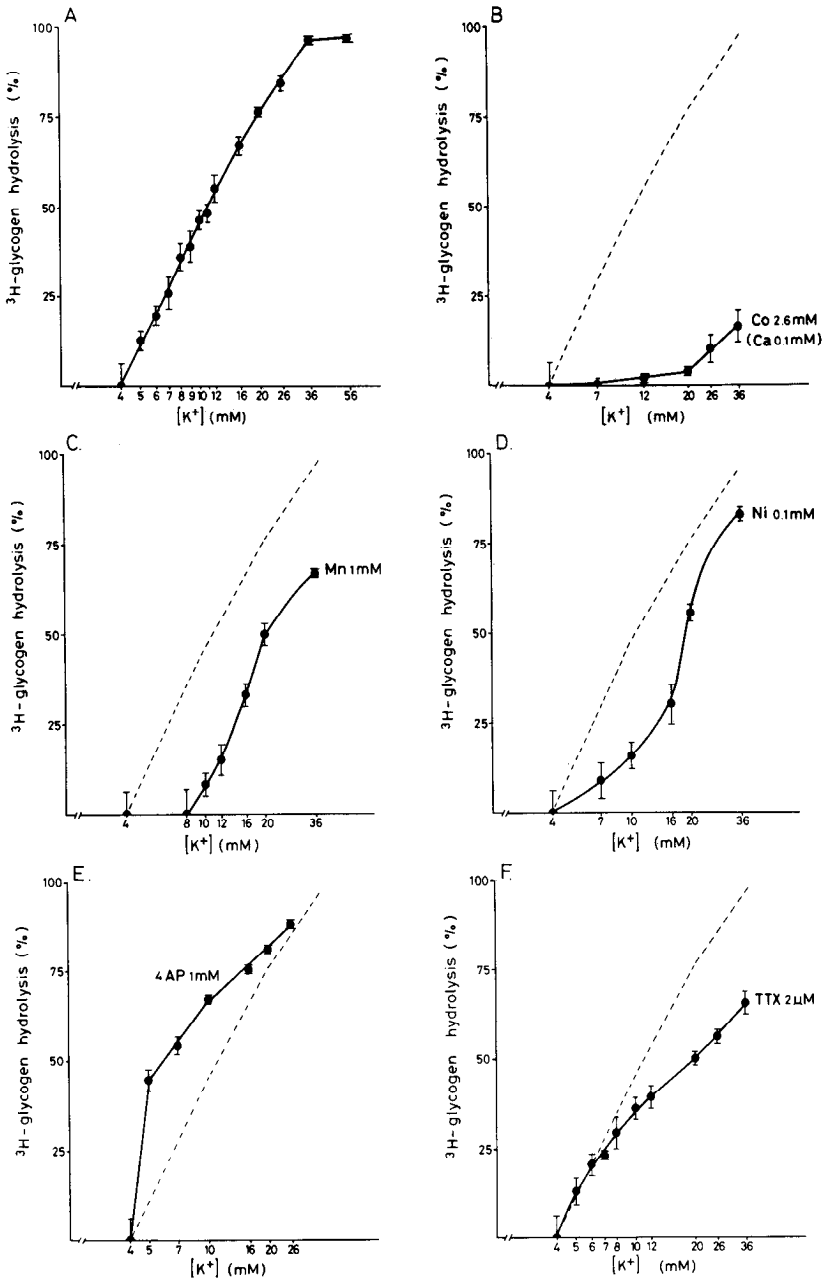

Figure 1. Effect of various pharmacological treatments on the concentration–response curve of the glycogenolytic effect of K^+. *A*, Concentration–response curve of the glycogenolytic effect of K^+ alone. *B*, Effect of Co^{2+} in a medium containing 0.1 instead of 2.6 mM Ca^{2+} and with total replacement of sulfates and phosphates by chloride in order to prevent the precipitation of Co^{2+}. Under these conditions, basal ^{3}H-glycogen levels were decreased by 18.1 ± 4.6% (n = 12). In 2 separate control experiments, the effect of Ni^{2+} and Mn^{2+} on the glycogenolysis evoked by K^+ of 16 mM were examined in a sulfate- and phosphate-free medium (see Materials and Methods). Both divalent cations expressed a blocking activity identical to that displayed in normal buffer. Results were (% glycogenolysis ± SEM; n = 6–10): K^+, 16 mM = 66.52 ± 2.93; K^+, 16 mM + Ni^{2+}, 0.1 mM (in sulfate- and phosphate-free medium) = 34.24 ± 1.03, to be compared with K^+, 16 mM + Ni^{2+}, 0.1 mM (in normal buffer, i.e., *D*) = 29.96 ± 6.09; K^+, 16 mM + Mn^{2+}, 1 mM (in sulfate- and phosphate-free medium) = 40.80 ± 1.2 to be compared with K^+, 16 mM + Mn^{2+}, 1 mM (in normal buffer, i.e., *C* = 33.09 ± 3.1). In *B–F* the concentration–response curve of K^+ alone is represented, for reference, with a *dashed line*. In *C–F* the $[Ca^{2+}]_0$ was 2.6 mM. *C*, Effect of 1 mM Mn^{2+}. *D*, Effect of Ni^{2+} at 0.1 mM. *E*, Effect of 4-AP, 1 mM. *F*, Effect of TTX, 2 μM. Mouse cerebral cortical slices were incubated as described in Materials and Methods. Results are the mean ± SEM of 19 separate experiments. Eight to 35 determinations were made for each point in the curves, except for 56 mM in *A*, for which n = 5. Basal ^{3}H-glycogen levels were (in cpm/mg protein) 16,840 ± 2006; they varied by less than 9% in the presence of Mn^{2+}, Ni^{2+}, 4-AP, and TTX alone.

was removed and replaced with fresh KRG (300 μl). The slice suspension was then sonicated for 5 sec, boiled for 10 min, and centrifuged again.

^{3}H-Glycogen was isolated from the supernatants by ethanol precipitation on filter paper, as previously described (Solling and Esmann, 1975; Quach et al., 1978; Magistretti et al., 1981). Aliquots (150 μl) of the deproteinized supernatants were pipetted on Whatman 31-ET filter paper discs (24 mm diameter) and immersed in an ice-cold 60% (vol/vol) ethanol/10% (wt/vol) trichloracetic acid solution (10 ml/filter) for 10 min. The filters were then placed in 66% ethanol solutions (10 ml/filter) at room temperature for 3 successive 20 min periods. After a final 5 min in acetone, filters were dried, placed into scintillation-counting vials, and counted for ^{3}H with an efficiency of 34%. Using this isolation technique, contamination by glucose was <0.5% and the glycogen recovery >99% (Magistretti et al., 1984).

Proteins were determined in the pellets, as described by Lowry et al. (1951).

6-^{3}H-Glucose (0.5 Ci/mmol) was purchased from Amersham International. Tetrodotoxin (TTX) and apamin were purchased from Sigma (St. Louis, MO), 4-aminopyridine (4-AP) and ouabain (g-strophantin) from Merck (Darmstadt, FRG), potassium isethionate from Eastman Kodak (Rochester, NY), and $NiCl_2$ from Fluka (Buchs, Switzerland). ω-Conotoxin was purchased from Peninsula Laboratories (St. Helens, UK); nifedipine, diltiazem, and Bay K 8644 were from Bayer (FRG). Verapamil was from Knoll (Liestal, Switzerland). All other compounds were purchased from Merck (Darmstadt, FRG).

Statistical analysis was performed by Student's t test.

Results

When mouse cerebral cortical slices are incubated in the presence of ^{3}H-glucose, a time-dependent incorporation into ^{3}H-glycogen occurs, reaching a plateau after approximately 30 min (Quach et al., 1978; Magistretti et al., 1981). We therefore examined the effect of K^+ on ^{3}H-glycogen levels at this point in time. As shown in Figure 1*A*, K^+ promotes a concentration-dependent hydrolysis of newly synthesized ^{3}H-glycogen, with an EC_{50} of approximately 11 mM and a maximal effect reached at 36 mM.

These results were obtained by exposing the slices to K^+ for 10 min. We therefore examined the time course of the glyco-

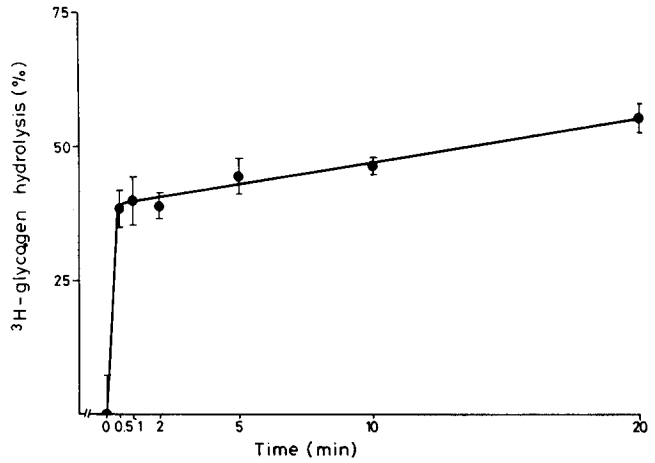

Figure 2. Time course of the glycogenolytic effect of K^+, 10 mM. Mouse cerebral cortical slices were incubated as described in Materials and Methods. Results are the mean ± SEM of 8–22 determinations from 7 separate experiments. Basal ^{3}H-glycogen levels were (in cpm/mg protein) 20,543 ± 2638.

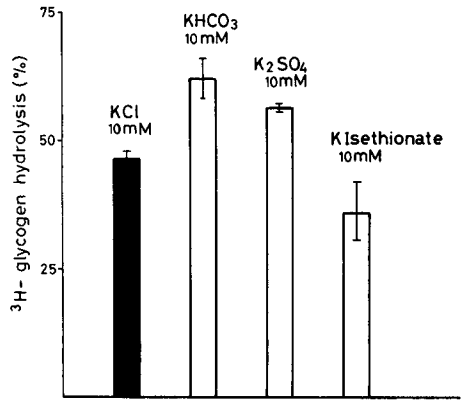

Figure 3. Glycogenolytic effect of various K^+ salts at a 10 mM concentration. Mouse cerebral cortical slices were incubated as described in Materials and Methods. Results are the mean ± SEM of 7–11 determinations from 4 separate experiments. Basal ^{3}H-glycogen levels were (in cpm/mg protein) 16,917 ± 2680.

genolytic effect of a fixed concentration of K^+, i.e., 10 mM. As shown in Figure 2, 71% of the maximal effect is achieved within 30 sec after the application of K^+, the shortest time that was compatible with the experimental paradigm.

In the experiments described above, the extracellular K^+ concentration was raised to the desired value by adding K^+ in the form of the KCl salt. Thus, a possible effect of osmolarity changes, particularly at high KCl concentrations, had to be considered. We therefore designed experiments in which NaCl was removed in a 1:1 ratio with the KCl added to maintain the total chloride concentration at 120 mM. As shown in Table 1, a decrease in NaCl to 90 mM slightly increased ^{3}H-glycogen synthesis; however, upon addition of 26 mM KCl to reach a total K^+ concentration of 30 mM (since the medium already contained 3 mM as KCl and 1 mM as KH_2PO_4; see Materials and Methods), the ^{3}H-glycogen hydrolysis reached 92.7%, a value virtually identical to that achieved when KCl was added in a medium containing 120 mM NaCl. Furthermore, the fact that the glycogenolytic effect of KCl was due to K^+ and not Cl^- could be verified by examining the effect of various potassium salts. As shown in Figure 3, $KHCO_3$, K_2SO_4, and K-isethionate at 10 mM elicited a glycogenolytic effect comparable to that achieved by KCl at 10 mM. We have also observed that Rb^+, an ion that has effects similar to K^+ on the resting membrane potential, is glycogenolytic as well (Fig. 4) in a Mn^{2+}-sensitive manner (see below for rationale of Mn^{2+}).

Table 1. Effect of decreasing NaCl concentrations on ^{3}H-glycogen levels and on the glycogenolytic action of K^+

NaCl (mM)	KCl (mM)	^{3}H-Glycogen levels (cpm/mg protein)	^{3}H-Glycogen hydrolysis (%)
120	—	16,603 ± 1393 (8)	—
120	10		49.02 ± 4.77 (10)
120	30		97.02 ± 0.1 (5)
110	—	17,574 ± 1208 (8)	—
110	10		47.95 ± 1.83 (7)
90	—	19,949 ± 1051[a] (7)	—
90	30		92.7 ± 1.05 (8)

Experimental conditions as in Figures 1–4, described in detail in Materials and Methods. Results are the mean ± SEM of the number of determinations indicated in parentheses from 2 separate experiments.

[a] Significantly different from ^{3}H-glycogen levels observed in KRG containing 120 mM NaCl ($p < 0.05$).

In a second phase of experimentation, we attempted to determine the mechanism by which an increase in $[K^+]_0$ elicits the hydrolysis of glycogen. We first examined the role of extracellular Ca^{2+} by decreasing $CaCl_2$ concentration to 0.1 mM and replacing it by $CoCl_2$, 2.6 mM, in the medium. As shown in Figure 1*B*, this manipulation blocked the glycogenolytic action of K^+ up to a concentration of 20 mM of the ion; at 26 and 36 mM K^+, an hydrolysis of glycogen was observed, although extremely reduced in comparison to control conditions. As shown in Figure 1, *C, D*, the K^+-evoked glycogenolysis was also inhibited when the effects of K^+ were tested in the presence of the reversible Ca^{2+}-channel blockers Mn^{2+} at 1 mM and Ni^{2+} at 0.1 mM. These experiments were performed in a medium containing 2.6 mM $CaCl_2$. Basal ^{3}H-glycogen levels were decreased by 18.1% when $CaCl_2$ was replaced with $CoCl_2$, but were only marginally affected by the addition of Mn^{2+} or Ni^{2+} in the presence of normal Ca^{2+} concentrations (see legend for Fig. 1). The K^+-evoked glycogenolysis was not blocked, but was in fact potentiated by Cd^{2+} at 20 μM (Table 2).

The effect of other Ca^{2+}-channel blockers selective for certain channel subtypes was also examined. Thus the dihydropyridine nifedipine at 10 μM, as well as diltiazem at 20 μM and verapamil at 10 μM, did not antagonize the glycogenolysis evoked by K^+ at 10 mM (Table 2). In fact, nifedipine per se displayed a slight glycogenolytic action that resulted in an additive effect with K^+ at 10 mM (Table 2). The selective blocker of N and L Ca^{2+}-channel subtypes ω-conotoxin (see Discussion) at 10 μM was also without antagonistic action on the K^+-evoked glycogenolysis (Table 2). Finally the Ca^+-channel agonist Bay K 8644 at 10 μM did not enhance the effect of K^+ on ^{3}H-glycogen levels (Table 2).

We also examined the effect of various K^+-channel blockers, such as tetraethylammonium (TEA), Ba^{2+}, Cs^+, 4-AP, and apamin. Ba^{2+}, Cs^+ at 1 mM, and TEA at 10 mM produced a small glycogenolytic effect when tested alone; TEA potentiated the ^{3}H-glycogen hydrolysis elicited by K^+ at 10 mM, whereas Ba^{2+}

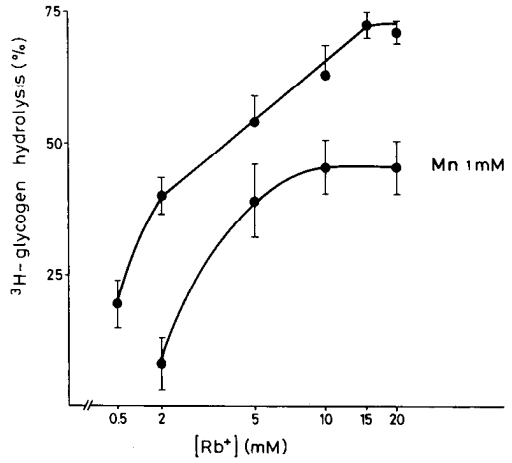

Figure 4. Concentration–response curve of the glycogenolytic effect of Rb^+ in the presence and absence of Mn^{2+}, 1 mM. Mouse cerebral cortical slices were incubated as described under Materials and Methods. Results are the mean ± SEM of 7–12 determinations from 4 separate experiments. Basal ^{3}H-glycogen levels (in cpm/mg protein), 34,159 ± 3403.

and Cs^+ were without effect (Table 3). As shown in Figure 1*E*, 4-AP at 1 mM shifted the concentration–response curve of K^+ markedly to the left, in particular for concentrations of K^+ ranging between 5 and 10 mM, without influencing basal ^{3}H-glycogen levels (see legend to Fig. 1). In contrast, apamin at 1 nM and 1 μM did not influence the K^+-evoked glycogenolysis (Table 3).

The effect of the selective Na^+-channel blocker TTX was also examined. As shown in Figure 1*F*, TTX at 2 μM did not significantly influence the glycogenolytic response of K^+ up to 8 mM, but gradually shifted to the right the concentration–response curve of higher K^+ concentrations. When tested alone, TTX did not significantly affect basal ^{3}H-glycogen levels (see legend for Fig. 1).

A mechanism accounting, to a small degree, for the clearance of increased $[K^+]_0$ is the activation of the Na^+/K^+-ATPase (Ballanyi et al., 1987, and references therein), an energy-consuming cellular process. The activity of the Na^+/K^+-ATPase can be blocked by the cardiac glycoside ouabain (Skou, 1965). We have therefore examined the effect of ouabain on the K^+-evoked glycogenolysis. As shown in Table 4, ouabain at 10 μM, a concentration of the cardiac glycoside not affecting ^{3}H-glycogen levels per se, partially inhibited the effect of K^+.

Discussion

In the series of experiments reported in this paper, we have observed that increases in $[K^+]_0$ promote a concentration-dependent hydrolysis of ^{3}H-glycogen newly synthesized by mouse cerebral cortical slices. This glycogenolysis is time-dependent. Pharmacological manipulations that prevent the movement of Ca^{2+} into the intracellular compartment effectively block the K^+-evoked glycogenolysis. These results suggest that the increase in $[K^+]_0$, which will bring the membrane potential to less negative values, triggers the opening of voltage-sensitive Ca^{2+} channels through which Ca^{2+} can enter and activate glycogenolysis. At the same time, the slight depolarization resulting from the increases in $[K^+]_0$ could enhance the occurrence of spontaneous action potentials. Each action potential in itself will in turn be accompanied by an entry of Ca^{2+} through voltage-sensitive Ca^{2+} channels. Direct measurements of $[Ca^{2+}]_i$ with the quin2 fluorescence technique in dispersed mouse embryonic brain cells have indicated that increases by 5–10 mM in $[K^+]_0$ significantly raise $[Ca^{2+}]_i$ (Morris et al., 1987). However, at least 2 questions should be addressed: first, how can an increase in free intracellular Ca^{2+} bring about the hydrolysis of glycogen; second, what is the subtype of voltage-sensitive Ca^{2+} channel that is activated?

The final step in the cascade of intracellular events that triggers

Table 2. Effect of various Ca^{2+}-channel blockers and of Bay K 8644 on the glycogenolytic action of K^+

Agent(s) added	^{3}H-Glycogen hydrolysis (%)
None	0.00 ± 4.91
K^+, 10 mM	51.53 ± 1.68
Nifedipine, 10 μM	19.06 ± 3.91[a]
Nifedipine, 10 μM + K^+, 10 mM	73.90 ± 1.92
Diltiazem, 20 μM	2.24 ± 1.19
Diltiazem, 20 μM + K^+, 10 mM	55.60 ± 1.58
Verapamil, 10 μM	8.86 ± 3.05
Verapamil, 10 μM + K^+, 10 mM	54.26 ± 1.95
ω-Conotoxin, 10 μM	3.93 ± 2.18
ω-Conotoxin, 10 μM + K^+, 10 mM	54.90 ± 1.98
Cd^{2+}, 20 μM	6.10 ± 0.17
Cd^{2+}, 20 μM + K^+, 10 mM	72.87 ± 5.36[b]
Bay K 8644, 10 μM	9.63 ± 2.76
Bay K 8644, 10 μM + K^+, 10 mM	57.78 ± 2.30

Experimental conditions as in Figures 1–4, described in detail in Materials and Methods. Results are the means ± SEM of 6–17 determinations from 4 separate experiments and are expressed as percentages of basal ^{3}H-glycogen hydrolyzed. Basal ^{3}H-glycogen levels were 19,620 ± 1855 cpm/mg protein. The effects of Cd^{2+} were tested in a sulfate- and phosphate-free medium (see Materials and Methods).

[a] Significantly different from ^{3}H-glycogen hydrolysis in the absence of added agents ($p < 0.05$).

[b] Significantly different from K^+ alone ($p < 0.001$).

Table 3. Effect of various K^+-channel blockers on the glycogenolytic action of K^+

Agent(s) added	^{3}H-Glycogen hydrolysis (%)
None	0.00 ± 7.25
K^+, 10 mM	46.60 ± 1.58
Cs^+, 1 mM	17.82 ± 5.18[a]
Cs^+, 1 mM + K^+, 10 mM	54.32 ± 4.24
Ba^{2+}, 1 mM	18.76 ± 2.89[b]
Ba^{2+}, 1 mM + K^+, 10 mM	44.29 ± 2.75
TEA, 10 mM	15.17 ± 4.95[a]
TEA, 10 mM + K^+, 10 mM	79.67 ± 3.40[c]
Apamin, 1 nM	10.34 ± 3.93
Apamin, 1 nM + K^+, 10 mM	55.05 ± 2.74
Apamin, 1 μM	9.63 ± 3.24
Apamin, 1 μM + K^+, 10 mM	53.7 ± 1.87

Experimental conditions as in Figures 1–4, described in detail in Materials and Methods. Results are the means ± SEM of 6–12 determinations from 11 separate experiments and are expressed as percentages of basal ^{3}H-glycogen hydrolyzed. Basal ^{3}H-glycogen levels were 19,960 ± 2072 cpm/mg protein.

[a] Significantly different from ^{3}H-glycogen hydrolysis in the absence of added agents ($p < 0.01$).

[b] Significantly different from ^{3}H-glycogen hydrolysis in the absence of added agents ($p < 0.005$).

[c] Significantly different from 10 mM K^+ alone ($p < 0.001$).

Table 4. Effect of ouabain on the glycogenolytic action of K^+

Agent(s) added	^{3}H-Glycogen hydrolysis (%)
None	0.00 ± 2.3
Ouabain, 10 μM	2.60 ± 3.08
K^+, 10 mM	48.87 ± 1.40
K^+, 10 mM + ouabain, 10 μM	6.91 ± 2.42[a]
K^+, 20 mM	75.41 ± 2.03
K^+, 20 mM + ouabain, 10 μM	31.08 ± 2.25[a]
K^+, 50 mM	97.01 ± 0.32
K^+, 50 mM + ouabain, 10 μM	44.05 ± 3.37[a]

Experimental conditions as in Figures 1–4, described in detail in Materials and Methods. Results are the means ± SEM of 8–13 determinations from 4 separate experiments and are expressed as percentages of basal ^{3}H-glycogen hydrolyzed. Basal ^{3}H-glycogen levels were 32,236 ± 3152 cpm/mg protein.

[a] Significantly different from K^+ alone ($p < 0.001$).

glycogenolysis is the conversion of phosphorylase from *b* (less active) to *a* (active) form. This conversion occurs by means of a phosphorylation via phosphorylase kinase. Phosphorylase kinase, in turn, can be activated either by a cAMP-dependent protein kinase or directly by Ca^{2+} (Cohen, 1982). Therefore an increase in intracellular free Ca^{2+} concentration can trigger the hydrolysis of glycogen. In fact Ververken et al. (1982) have demonstrated a crucial role for Ca^{2+} in the activation of phosphorylase *b* in the cerebral cortex.

Three types of Ca^{2+} channels have recently been described; they are denominated T, N, and L, and present different electrical properties and pharmacological sensitivities (Nowycky et al., 1985; Fox et al., 1986, 1987; Tsien, 1987). The pharmacological sensitivity of each of the various channel subtypes is as follows: T channels are blocked by Ni^{2+} (80% block at 0.1 mM) but are weakly sensitive to Cd^{2+} and ω-conotoxin, and are insensitive to dihydropyridines; in contrast, N and L channels are only weakly sensitive to Ni^{2+}, but are blocked by Cd^{2+} and ω-conotoxin; in addition L channels are blocked by dihydropyridines and activated by Bay K 8644 (Fox et al., 1986, 1987; Tsien, 1987). The glycogenolytic effect of K^+ is inhibited by Ni^{2+} at 0.1 mM (Fig. 1*D*), but not by Cd^{2+} at 20 μM (Table 2), a concentration of the ion that almost completely blocks voltage-sensitive Ca^{2+} channels of the L and N subtype (Fox et al., 1987; Miller, 1987). Furthermore, the action of K^+ is insensitive to nifedipine, diltiazem, verapamil, Bay K 8644, and ω-conotoxin (Table 2). This pharmacological profile of blockade therefore strongly suggests that activation of T channels mediates the glycogenolytic effect of K^+. It should nevertheless be pointed out that the biochemically assessed responses observed in a preparation of cerebral cortical slices reflect the properties of an heterogeneous cell population; conclusions as to the involvement of channel subtypes should be taken with caution, awaiting confirmation in a homogeneous cell system.

The contribution of the voltage-sensitive Na^+ channels in membrane depolarization, and in the subsequent entry of Ca^{2+}, can be estimated to commence at 8 mM $[K^+]_o$, as indicated by the fact that the glycogenolytic effect of K^+ is unaffected by TTX up to this concentration.

The analysis of the effects of pharmacological agents known to block various types of K^+ channels can provide some further clues as to the mechanism of the K^+-evoked glycogenolysis. At least 5 voltage-dependent K^+ channels have been described: delayed-rectifier, Ca^{2+}-dependent (K_{Ca}), A-channel, M-channel, and inward-rectifier (Hille, 1984). A-channels are blocked by low millimolar concentrations of 4-AP, and to a considerably lesser degree, by TEA (Thompson, 1977). Analysis of their kinetics has indicated that they very rapidly activate following depolarizations ranging from −60 to −45 mV (Connor and Stevens, 1971; Neher, 1971); they also rapidly inactivate with maintained depolarization. These channels therefore play a major role controlling excitability at voltages near the resting potential. They, in fact, prevent small depolarizing stimuli from reaching threshold since, when activated, they repolarize briefly (because of their rapid inactivation) the membrane potential (Rogawski, 1985). Interestingly, 1 mM of 4-AP dramatically enhances the glycogenolytic effect of low K^+ concentrations between 5 and 10 mM (i.e., small depolarizing stimuli), whereas its effect fades off at K^+ concentrations above 16 mM (Fig. 1*E*). These observations would imply that, in the absence of 4-AP, the depolarizations brought about by low K^+ concentrations and the Ca^{2+}-mediated glycogenolysis that would subsequently occur are dampened by the activation of transient outward A-currents. Blockade of A-channels by 4-AP would remove this shunt and allow for the full expression of the glycogenolytic action of low K^+ concentrations. The insensitivity of the K^+-evoked glycogenolysis to apamin, a K_{Ca} blocker, and to Ba^{2+} and Cs^+ (Table 3), 2 blockers of the delayed- and inward-rectifiers (Hille, 1984), further stresses the specificity of 4-AP on the A-channels. It should, however, be noted that 4-AP has been shown by Rogawski and Barker (1983) to directly increase Ca^{2+} influx into neurons. However, the fact that the action of 4-AP is expressed only in the presence of increased $[K^+]_o$ (Fig. 1*E*) would tend to discard the notion of a direct action of 4-AP on Ca^{2+} channels.

The clearance of $[K^+]_o$ is predominantly undertaken by glial elements in the vicinity of active neurons (Orkand et al., 1966; Kuffler, 1967; Sykova, 1983, and references therein). Two main mechanisms appear to exist for the restoration of basal $[K^+]_o$: (1) a Ba^{2+}-sensitive passive uptake into glial cells, and (2) an ouabain-sensitive active transport through the Na^+/K^+-ATPase (Sykova, 1983). The Ba^{2+}-sensitive K^+ uptake would occur through spatial buffer currents, whereby K^+ is taken up at sites of increased neuronal activity and, through the electrically coupled glial syncytium, would be released at regions where $[K^+]_o$ is not increased (Gardner-Medwin, 1983; Immel and Steinberg, 1986). Very recently, elegant experiments by Ballanyi et al. (1987) have demonstrated in guinea pig olfactory cortex slices that the ouabain-sensitive K^+ uptake contributes only marginally to $[K^+]_o$ clearance. Furthermore, Gardner-Medwin (1983) has demonstrated that $[K^+]_o$ changes are not dependent on metabolism, as they are unaffected by ouabain or by metabolic inhibitors such as 2,4-dinitrophenol or iodoacetate. In view of the foregoing, it seems unlikely that the K^+-evoked glycogenolysis could be the consequence of the activation of an energy-consuming process such as an increased activity of the Na^+/K^+-ATPase. One possible way to test this hypothesis was to examine the potential inhibitory action of ouabain on the K^+-evoked glycogenolysis. As shown in Table 4, ouabain partially inhibited the effect of K^+; however this experiment did not provide a definitive answer, since we had previously demonstrated that the glycogenolytic action of vasoactive intestinal peptide and adenosine, 2 agents that are not known to stimulate the Na^+/K^+-ATPase in the CNS, is antagonized by ouabain (Magistretti et al., 1986). In fact, antagonism of glycogenolysis appears to

be a general property of ouabain, most likely unrelated to its inhibitory effect on the Na^+/K^+-ATPase, since the cardiac glycoside also antagonizes the hormone-stimulated glycogenolysis in adipocytes (Ho and Jeanrenaud, 1967) and stimulates glycogen synthesis in adipocytes (Ho and Jeanrenaud, 1967) and in skeletal muscle (Clausen, 1966).

Some comments should be presented as to the possible cellular localization of the K^+-evoked glycogenolysis. Thus, Pumain and Heinemann (1985) have shown that the increase in $[K^+]_0$ and the parallel decrease in $[Ca^{2+}]_0$ that follow the iontophoretic application of glutamate and aspartate in rat neocortex occur predominantly in layers II–III and V, suggesting that the "Ca^{2+} sinks" may be the cell bodies of pyramidal neurons. This view was substantiated by the observation that, following degeneration of the pyramidal tract, the maximum Ca^{2+} changes were considerably reduced and the laminar pattern of the $[Ca^{2+}]_0$ decrease disappeared (Pumain and Heinemann, 1985). Since the K^+-evoked glycogenolysis appears to be mediated by an inflow of Ca^{2+} in the target cells, it is conceivable that this metabolic action of K^+ in the neocortex occurs in neurons—in pyramidal cells in particular. This view would be supported by the fact that voltage-sensitive Ca^{2+} channels of the T subtype have been demonstrated in neurons (Nowycky et al., 1985; Fox et al., 1986, 1987; Tsien, 1987). It should nevertheless be noted that voltage-sensitive Ca^{2+} channels of an undefined subtype have been demonstrated in cultured astrocytes (MacVicar, 1984). However, the fact that these channels were blocked by Cd^{2+}, an ionic species to which T channels and K^+-evoked glycogenolysis are insensitive, would suggest that they were of the N or L, or possibly even of a yet unrecognized, subtype. Furthermore, we have previously demonstrated that, in contrast to the vasoactive intestinal peptide norepinephrine and adenosine, increases in $[K^+]_0$, even at concentrations as high as 50 mM, only marginally stimulate glycogenolysis in primary cultures of astrocytes (Magistretti et al., 1983). This set of observations would therefore suggest that the K^+-evoked glycogenolysis occurs predominantly in neurons.

The results presented in this paper further delineate the role of K^+ in intercellular communication. They suggest that K^+, released from active neurons, could mobilize energy substrates in regions of increased neuronal activity, where the $[K^+]_0$ can reach values up to 10–12 mM; these are concentrations at which K^+ promotes a significant glycogenolysis.

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