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Modulation of Voltage-Gated Potassium Channels in Human T Lymphocytes by Extracellular Glutamate

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ABSTRACT

Glutamate is present in the plasma under tightly regulated concentrations. However, under conditions of immune deficiency, such as AIDS and malignancy, its plasma levels are highly elevated. In vitro, glutamate interacts with T lymphocytes, affecting mitogen-induced calcium responses, whereas at high doses, it impairs T lymphocyte proliferation, a process strongly dependent on the activity of voltage-gated potassium channels. In this study, we demonstrate novel dose-related effects of the endogenous ligand glutamate and its metabotropic and non-*N*-methyl-D-aspartic acid receptor agonists on the electrophysiological properties of native $K_v1.3$ channels of human T lymphocytes. Glutamate, at concentrations within normal plasma levels, positively modulates $K_v1.3$ channel gating, causing currents to activate faster and at significantly more hyperpolarized potentials, hence rendering the T lymphocyte readily responsive to immune stimuli. This effect is maximal at 1 μM Glu and is fully mimicked by a 100 μM concentration of the metabotropic receptor agonist *trans*-(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid. Most importantly, Glu, at concentrations $\geq$100 μM, which in vitro produce suppression of mitogen-induced proliferation, significantly decreases whole-cell potassium currents by increasing current and steady-state inactivation. This effect saturates at 1000 μM and seems to result from the subsequent activation of low-affinity metabotropic Glu receptors, as suggested by specific agonist data. Therefore, the antiproliferative effects of high glutamate may, at least in part, result from its inhibitory effect on the potassium current, suggesting an in vivo immunosuppressive role of elevated plasma glutamate.

It is well established by now that neurotransmitters and neuropeptides, apart from their signaling role in the CNS, can also directly influence T lymphocyte functions (Levite, 2000). Glu, the major excitatory neurotransmitter in the CNS, is also present in the plasma of healthy persons, under tightly regulated concentrations (10–50 μM) (Divino Filho et al., 1998; Graham et al., 2000), and seems to play a role in the immune system as well. Evidence for this comes from a significant number of clinical studies consistently showing strong correlation between elevated plasma Glu (well above 100 μM) and conditions of immune deficiency, such as AIDS (Eck et al., 1989; Droge at al., 1993; Ferrarese et al., 2001) and malignancies (Ollenschlager et al., 1989; Eck et al., 1990). An immunosuppressive role of elevated Glu is further supported by in vitro data showing that preincubation of T lymphocytes with concentrations of Glu above 100 μM significantly inhibits mitogen-induced T lymphocyte proliferation (Droge et al., 1988; Sommer et al., 1994). Lombardi et al. (2001) showed that, at the same doses at which it suppresses T-cell proliferation, Glu hinders the sustained calcium response that follows the stimulation of the cell and is absolutely necessary for activation and cell cycle progression. These results suggest that the antiproliferative effects of Glu are caused by an interference with the intracellular calcium signal. In the same report, it was also demonstrated that Glu, at doses below normal plasma levels, maximally enhances this calcium response. However, despite all the in vivo and in vitro evidence that point to an immunoregulatory role of Glu, the underlying target(s) and mechanism(s) remain largely unknown. Therefore, the need to clarify the nature of interaction between Glu and T lymphocytes is of obvious biological and clinical importance.

The fact that Glu affects the sustained phase of intracellular Ca^{2+} levels and thus calcium-dependent processes,

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ABBREVIATIONS: CNS, central nervous system; CRAC, calcium-release activated calcium channel; *trans*-ACPD, *trans*-(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid; NMDA, *N*-methyl-D-aspartic acid; MgTx, margatoxin; GDPβS, guanosine 5′-[β-thio]diphosphate; GTPγS, guanosine 5′-[γ-thio]triphosphate; (*S*)-DHPG, (*S*)-3,5-dihydroxyphenylglycine; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)-glycine; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; g-V, conductance-to-voltage curves.

such as activation and proliferation, points to cellular components that take part in the mediation and regulation of this phase. Antigen receptor stimulation causes an initial transient intracellular Ca^{2+} elevation that leads to the opening of the calcium-release activated calcium (CRAC) channels and to the subsequent influx of Ca^{2+} (Berridge et al., 2000). However, for Ca^{2+} to flow through CRAC channels into the cell, it requires a negative membrane potential (hyperpolarization) as its driving force. In T lymphocytes, this electromotive force is initially provided by K_v channels (Lin et al., 1993) and, at later stages, by the calcium-dependent potassium channels (Ghanshani et al., 2000). Thus, whereas CRACs mediate the Ca^{2+} influx, K^+ channels act as the regulator of the amplitude and duration of the Ca^{2+} signal.

In an effort to find a molecular link between extracellular Glu and T lymphocyte function, we postulated on an interaction between Glu and the key regulator of the resting and activating state of T lymphocytes, namely the $K_v1.3$ channel (Cahalan et al., 2001).

We were led to this hypothesis based on two facts: 1) that the antiproliferative effects of Glu were only seen if Glu was added to the medium before the stimulant (Lombardi et al., 2001), pointing to the early events of activation, therefore making the involvement of CRAC channels less probable because they need to be functional during the whole process, and 2) that at the early events of T-lymphocyte activation and proliferation, the membrane hyperpolarization needed for Ca^{2+} influx is strongly dependent on K_v channel activity (Hess et al., 1993; Verheugen et al., 1997).

To investigate the above hypothesis, $K_v1.3$ current characteristics were directly measured in the absence and presence of Glu using the patch-clamp technique (Hamill et al., 1981). Our data demonstrate that Glu has a dual effect on K_v currents, depending on its extracellular concentration. On the one hand, at extracellular concentrations coinciding with normal plasma levels, Glu facilitates $K_v1.3$ channel activation, whereas, on the other hand, at concentrations similar to the ones found in plasma at conditions of immune deficiency, it substantially decreases $K_v1.3$ currents, an effect known to suppress T cell responses (Chandy et al., 1984; DeCoursey et al., 1984; Shah et al., 2003) and to lead to immunosuppression (Koo et al., 1997; Chandy et al., 2004). Furthermore, we present evidence that T lymphocytes express functional Glu receptors with metabotropic pharmacology, in addition to the previously identified ionotropic Glu receptors (Lombardi et al., 2001; Ganor et al., 2003), whose activation leads to $K_v1.3$ current modulation.

Materials and Methods

Chemicals and Salts. Ficoll-Hypaque (Histopaque-1077), glutamate, *trans*-ACPD, NMDA, MgTx, 4-aminopyridine, GDPβS, and GTPγS were obtained from Sigma-Aldrich (St Louis, MO). DCG-IV and (*S*)-DHPG were obtained from Tocris Cookson (Bristol, UK). RPMI 1640 medium was purchased from Biochrom (Berlin, Germany). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) and kainate were kindly provided by Dr. L. M. Nowak. (Cornell University, Ithaca, NY) Salts were of analytical grade and obtained from Fluka Biochemica (Buchs, Switzerland).

Cell Isolation. Human peripheral T lymphocytes were obtained from 14 consenting healthy volunteers on the day of the experiment. Peripheral mononuclear cells were separated from heparinized whole blood by standard Ficoll-Hypaque density gradient centrifugation (Boyum, 1968). Monocytes were depleted by adhesion to plastic tissue culture flasks as described previously (Verheugen and Korn, 1997). The resulting cell population contained >80% T lymphocytes, as determined by anti-CD3 staining in fluorescence-activated cell sorting measurements. The purified T lymphocytes were kept in RPMI 1640 medium for use on the same day and were extensively washed with Glu-free recording solution before each experiment.

Solutions. The Glu-free extracellular solution used in patch-clamp experiments contained 135 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, pH 7.4; osmolality, 280 mOsmol/kg). Solutions were applied by the Superfast 8 perfusion system (List Medical, Darmstadt, Germany) and driven away with the use of a peristaltic pump (List Medical). The pipette solution was composed of 100 mM KCl, 40 mM KF, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES, pH 7.4; osmolality, 290 mOsmol/kg). The estimated free Ca^{2+} concentration of the pipette solution was 100 nM, well below the threshold of K_{Ca} channel activation (Cahalan et al., 2001).

Electrophysiological Recordings. Membrane currents were measured in the whole-cell configuration of the patch-clamp technique using an Axopatch 100B (Axon Instruments, Union City, CA) amplifier, interfaced to a personal computer with a Digidata 1200 (Axon Instruments) analog-to-digital converter. Seal resistances of 10 to 20 GΩ were obtained after slight suction was applied to the interior of the pipette. Membrane voltages were corrected for liquid junction potentials. No correction for leak currents was performed. Patch pipettes were fabricated from R-series Borosilicate Glass Capillaries (World Precision Instruments, Sarasota, FL) using a two-stage puller (L/M-3P-A; List Medical) and had resistances between 3 and 5 MΩ. All experiments were performed at room temperature (20–25°C). Currents were low-pass filtered at 2 kHz.

Voltage Protocols. Cells were clamped at a holding potential of −90 mV and were constantly perfused by Glu-free solution, unless otherwise stated. Kinetic parameters were calculated by fitting whole-cell currents to the Hodgkin-Huxley n^4j kinetic model (Cahalan et al., 1985). Peak currents were measured in response to 1000-ms voltage steps from −80 to + 70 mV in 15 mV increments, given every 60 s, and conductance-to-voltage curves (g-V) were constructed using the chord equation (Hille, 2001). The voltage dependence of steady-state inactivation was estimated by clamping the cell membrane at −90 mV and then stepping to various prepulse potentials (−120 to 0 mV, in 15-mV increments) for 100 s and then to a test pulse of +40 mV for 100 ms.

Data Analysis. Current measurements were performed using the pClamp software (ver. 6.3; Axon Instruments). Data were further analyzed using Origin technical graphics and data analysis program (OriginLab Corp., Northampton, MA). Peak conductance-to-voltage and steady-state inactivation curves were fitted to Boltzmann functions. The statistical significance of average values between control and Glu-treated cells was assessed using the Student's t test with an accepted level of $p < 0.05$. The goodness of data fit to exponential or Boltzmann functions was evaluated with the Hamilton's R coefficient.

Results

Identification of Whole-Cell $K_v1.3$ Currents. Using the patch-clamp technique, we recorded whole-cell potassium currents in freshly isolated human T lymphocytes from 14 consenting healthy subjects. Currents were recorded only after their constancy was confirmed by successive depolarizing steps of 200 ms to −20 or +40 mV, given every 60 s. Most cells presented stable currents 15 min after going whole-cell (Fig. 1A). Currents were characterized based on their ionic selectivity, pharmacology, kinetics and activation characteristics. Analysis of a series of tail current protocols (data not shown) showed that the recorded currents had a reversal

potential of −76 mV. This value is very close to the calculated potassium (K^+) reversal potential ($EK^+ = -85.3$) for our recording solutions and away from that of chloride (Cl^-) ($E_{Cl^-} = -8.4$ mV), indicative of a K^+-selective conductance. These K_v currents were completely blocked by 5 nM MgTx (Garcia-Calvo et al., 1993) as well as by 5 mM 4-aminopyridine (DeCoursey et al., 1984), consistent with their being $K_v1.3$. Activation of K_{Ca} channels was avoided by keeping intracellular (pipette) free calcium concentration at 100 nM, well below the channel's activation requirement (Cahalan et al., 2001).

The recorded currents manifested cumulative inactivation, a characteristic of $K_v1.3$ channels. Double-pulse experiments to +40 mV with varying interpulse intervals showed that the time needed for the currents to completely recover from inactivation was 50 s. Thus, the activation characteristics of these currents were evaluated by a sequence of step depolarizations (−80 to +70 mV) every 60 s, to avoid carried-over inactivation. Current responses remained stable for more than an hour, as determined by repeatedly subjecting a number of cells ($n = 7$) to the above protocol. The average peak current at +40 mV was 420 ± 150 pA (data collected from 40 cells). Figure 1C1 shows IK_v responses to depolarizations physiologically relevant for the T cell (−80 to −5 mV). Under these conditions, IK_v started to activate in response to a −35-mV depolarizing step (voltage activation threshold), and its amplitude increased in a voltage-dependent way.

Extracellular Glu Potentiates $K_v1.3$ Currents at Concentrations below or within Normal Plasma Levels. Application of 1 and 10 μM Glu to T lymphocytes, after 1 to 2 min, caused alterations in the macroscopic potassium current characteristics. These currents were abolished when 5

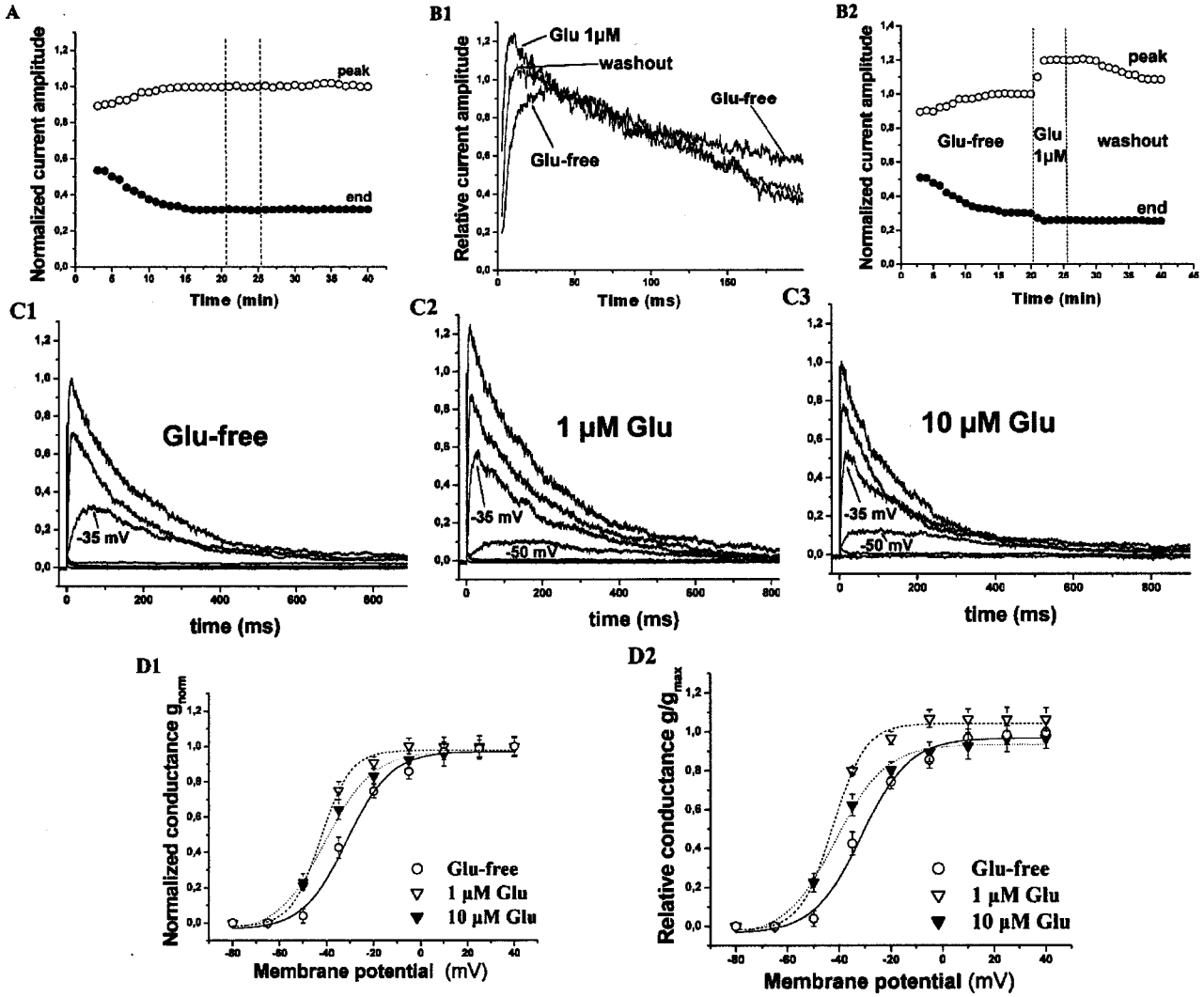

Fig. 1. Glutamate alters $K_v1.3$ current responses in human T lymphocytes. A, time course and stability of $K_v1.3$ currents after the transition of T lymphocytes to the whole-cell conformation. A cell constantly perfused with Glu-free solution was clamped to −90 mV and stepped every min to a test pulse of −20 mV for 200 ms. Peak (○) and end (●) amplitudes of the recorded currents show that a period of 15 min is necessary for the currents to stabilize. The vertical dotted lines refer to the times at which glutamate would be applied and removed from the bathing solution. B, addition of 1 μM Glu to the bath accelerates current activation rate and increases its peak amplitude (B1). Glutamate was applied 20 min after going whole cell to ensure $K_v1.3$ current stability (B2). The effect becomes maximal 2 min after Glu application and continues to persist despite prolonged washout with Glu-free solution (see data points in B2). The washout trace in figure B1 was recorded 30 min after removal of Glu. C, $K_v1.3$ currents recorded from a T-lymphocyte sequentially exposed to Glu-free solution (C1) and1 μM (C2) and 10 μM (C3) Glu. Current traces are in response to depolarizations from −80 to −5 mV. D, Glu shifts g-V to more negative potentials. Values are either normalized to the maximum conductance of each treatment (D1) or to the maximum conductance at Glu-free bath (D2). Each curve is the average of 11 different experiments and is fitted by a Boltzmann function. The midpoint of activation is −31.3 ± 1.2 mV, −44.6 ± 1.5 mV, and −42.6 ± 1.7 mV for Glu-free (○), 1 μM (▽), and 10 μM (▼) Glu, respectively.

nM MgTx was coapplied with Glu, supportive of an interference of extracellular Glu with the operation of the $K_v1.3$ channel. Washout of Glu for more than 15 min with Glu-free solution did not result in current recovery (Fig. 1, B1 and B2). Figure 1, C2 and C3, shows IKv responses (−80 to −5 mV) in the presence of 1 and 10 μM Glu of a T lymphocyte first bathed in a Glu-free solution (Fig. $1C_1$).

Comparison of IK_v before and after the addition of 1 and 10 μM Glu (Fig. 1, C2 and C3 versus C1, same cell) makes it obvious that current responses activate more quickly and at a more negative membrane potential (−50 mV versus −35 mV in Glu-free solution) and are enhanced in amplitude. It is interesting that the peak and sustained $K_v1.3$ currents are higher in 1 μM Glu ($n = 20$) compared with 10 μM ($n = 21$). The effects of Glu on IK_v were quantified by converting peak current amplitudes to peak conductance (g) values (see *Materials and Methods*) and plotted against their corresponding voltage (g-V curves) (Fig. 1, D1 and D2). In the presence of 1 and 10 μM Glu, the g-V relation is shifted toward more negative potentials by −13.3 ± 1.9 mV ($n = 20$; $p < 0.001$) and −11.3 ± 2.1 mV ($n = 21$; $p < 0.001$), respectively, compared with Glu-free solution ($n = 50$), as calculated by the midpoints of activation ($V_{1/2}$; voltage at which 50% of the channels are conducting). In the presence of 1 μM Glu, the g-V curve becomes steeper compared with Glu-free or 10 μM Glu; the corresponding values for the slope factor (k_n) of the g-V curve were 6.8 ± 0.9 and 9.6 ± 1.5 for 1 and 10 μM Glu, respectively, and 9.3 ± 1.4 for the Glu-free solution.

To investigate whether the increase in maximum current conductance and the hyperpolarizing shift in the activation curve of the K_v channel results from the stabilization of the channel into its open state by low doses of Glu, we studied tail currents from a protocol in which the membrane was first depolarized to a fixed potential of +40 mV (maximum activation) for 15 ms (before current inactivation ensues), followed by test pulses from −120 to +20 mV. At strong hyperpolarized test potentials (−120 to −70 mV), where the forward rate of activation is negligible, the decay of the tail currents (reflecting primarily the channel's closing rate) was unaffected by Glu. Thus, the hyperpolarizing shift seen in the presence of Glu is not caused by a slowing in the transition of open channels to their closed state.

Effects of Glu on $K_v1.3$ Current Kinetics. The above alterations of IK_v by Glu were accompanied by a significant increase in the activation rate of the current. The time needed for the current to reach its peak value after a depolarizing stimulus was prominently shorter in the presence of extracellular Glu (Fig. 1, C2 and C3), reflecting a faster entry of $K_v1.3$ channels to their open (conducting) state. This may explain the increased peak current amplitude observed in Glu versus Glu-free bath. Activation time constants (τ_n) calculated for $K_v1.3$ currents elicited at −20 mV were significantly ($p < 0.001$) smaller in 1 and 10 μM Glu [$\tau_n = 1.9 \pm 0.4$ ms ($n = 21$) and 2 ± 0.3 ms ($n = 21$), respectively], compared with Glu-free solution ($\tau_n = 3.5 \pm 0.5$ ms; $n = 21$). This effect was consistently present at all voltages tested and did not differ between Glu treatments (Fig. 2A).

Furthermore, when the concentration of Glu was raised from 1 to 10 μM, IK_v decayed at a faster rate. This reflects an increase in the transition rate of the open channel to its inactivated (nonconducting) state. The velocity of current decay is described by an exponential time constant (τ_j), calculated by fitting the currents with the n^4j model. Increase in the value of this constant represents slower current inactivation. For currents elicited by depolarizations to −20 mV, τ_j values were significantly ($p < 0.005$) smaller in the presence of 10 μM Glu ($\tau_j = 130.2 \pm 8.2$ ms; $n = 20$), compared with 1 μM Glu (161 ± 8 ms; $n = 21$) or with Glu-free solution (168.1 ± 9.6 ms; $n = 21$). The effect was consistent at all potentials tested (Fig. 2B). The acceleration in the inactivation rate in the presence of 10 μM Glu coincides with a decrease in the peak current amplitude compared with 1 μM Glu. Therefore, one might postulate that the decrease in peak current amplitude at 10 versus 1 μM Glu (Fig. 1, compare C3 and C2) was caused by the faster entry of activated channels to the inactivated state and thus by the masking of current enhancement readily observable at 1 μM Glu.

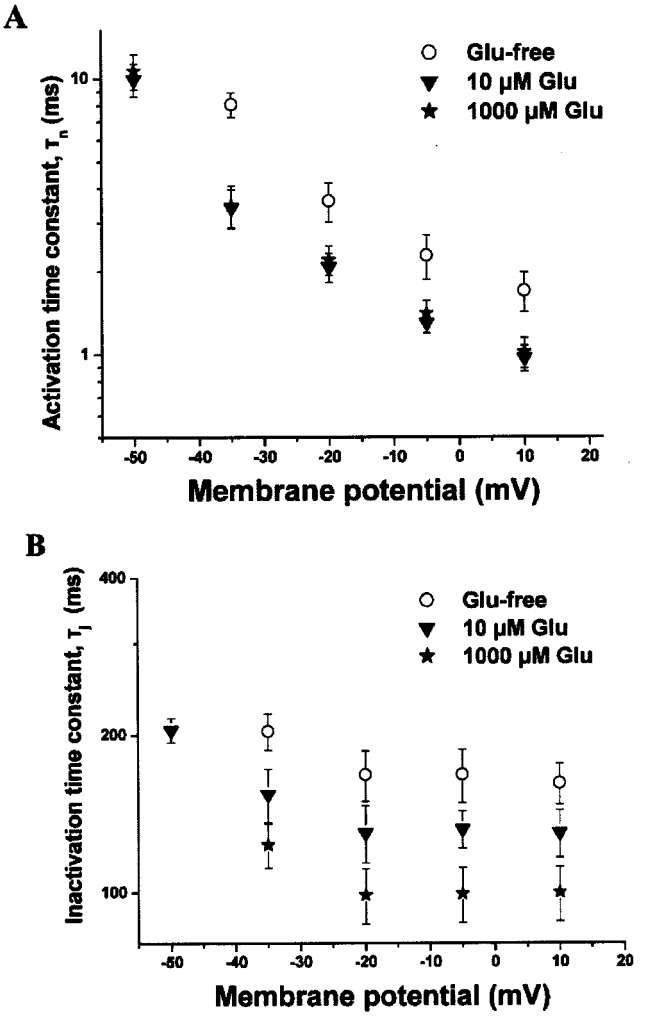

Fig. 2. Effects of Glu on Kv current gating kinetics. A, time constants of activation (τ_n) are plotted against different membrane potentials (−50 to 10 mV). In the presence of 10 μM (▼) or 1000 μM (★) Glu, τ_n values are significantly ($p < 0.001$) shorter compared with Glu-free extracellular solution (○). The activation rate is similar for both Glu concentrations, indicating that it remains unaffected by dose escalation. B, Glu enhances the rate of $K_v1.3$ current inactivation in a dose-dependent manner. Time constants of inactivation (τ_j) are plotted against the membrane potential of cells bathed in Glu-free extracellular solution (○), 10 μM (▼), and 1000 μM (★) Glu. Application of 10 μM of Glu causes a statistically significant ($p < 0.005$) decrease of τ_j constants for all potentials tested. Increasing Glu concentration to 1000 μM results in a marked decrease of τ_j compared with 10 μM of Glu ($p < 0.001$).

The differences between 1 and 10 μM Glu in current inactivation, amplitude, and voltage dependence led us to investigate the effects of higher Glu concentrations in the IK_v activation characteristics of T lymphocytes isolated from the same healthy subjects. This is of major importance because, as mentioned above, elevation of plasma Glu is strongly correlated to several conditions of immunodeficiency and is known to decrease both intracellular Ca^{2+} levels and T lymphocyte proliferation in vitro, with an as-yet-unexplained mechanism.

Suppression of K_v1.3 Current Caused by Elevation of Glu Concentration above Normal Plasma Levels. Exposure of T lymphocytes to increasing concentrations of Glu resulted in a proportionally faster and more pronounced decay of IKv. The effect was saturated at 1000 μM Glu because no further changes could be observed at higher concentrations. T lymphocytes bathed in Glu-free solution were sequentially exposed to increasing concentrations of Glu (10, 100, and 1000 μM), and were stepped to -20 mV and $+40$ mV every 60 s from a holding potential of -90 mV, to detect changes in gating kinetics near the midpoint of activation and maximal conductance. Figure 3A shows such IK_v traces in Glu-free, 10 μM Glu, and 100 μM Glu. Figure 3C1 shows the effects of increasing Glu concentrations on the IK_v amplitude in response to a -20-mV depolarizing pulse. Washout of 100 and 1000 μM Glu with Glu-free solution for 15 min resulted in a slow and incomplete recovery of the current from the inhibitory effects of the above Glu concentrations

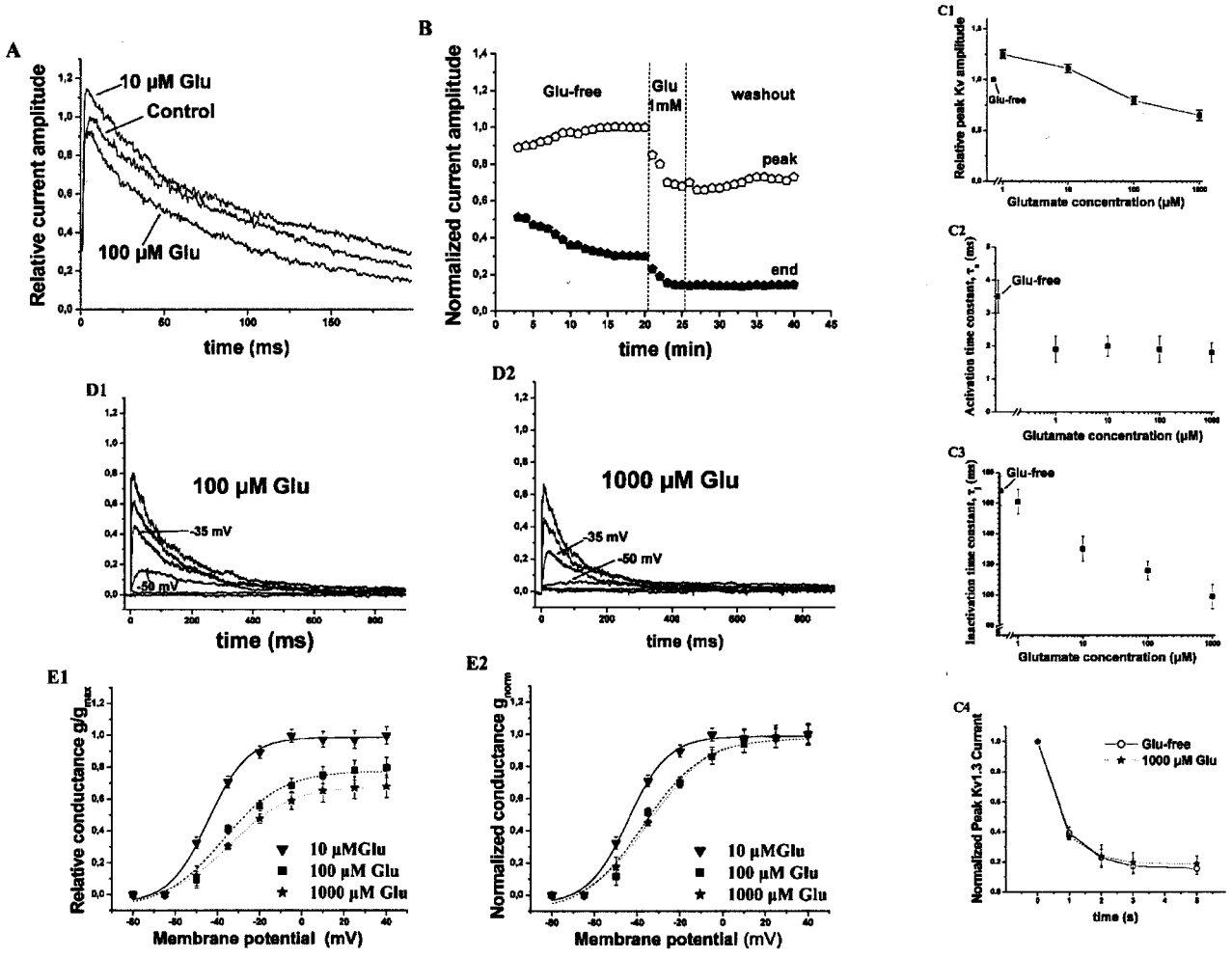

Fig. 3. Elevation of extracellular Glu to concentrations coinciding with those inhibiting T-lymphocyte proliferation decreases Kv current responses. A, current traces from T lymphocytes clamped at -90 mV and stepped to $+40$ mV for 200 ms every min. Increasing the concentration of Glu from 10 to 100 μM significantly enhanced the rate and extent of current inactivation without affecting current activation rate. In 100 μM Glu, current amplitude is decreased at the beginning as well as at the end of the pulse, compared with 10 μM Glu and Glu-free solution (A1). B, peak (○) and end (●) current amplitudes from a similar experiment during which a cell was stepped to -20 mV, a value near the midpoint of maximal K_v1.3 activation. Application of 1000 μM of Glu to the bath 20 min after transition to the whole-cell conformation, lead to a marked reduction of current that was not reversible even after prolonged washout. C, exposure of T lymphocytes to increasing Glu concentrations decreases the amplitude and accelerates the inactivation of K_v1.3 currents in a dose-dependent fashion without further affecting its rate of activation. Peak amplitudes (C1) and time constants of activation (C2) and inactivation (C3) for currents elicited during long depolarizing steps to -20 mV given every min, are plotted as a function of Glu concentration. Data shown are average of five separate experiments. Cumulative inactivation of K_v1.3 channels, studied by repetitive voltage pulses from -80 to $+30$ mV given every 1 s, was not affected by glutamate (C4). D, K_v current responses in the presence of 100 and 1000 μM Glu from a T lymphocyte sequentially bathed to 10, 100, and 1000 μM Glu. The current responses are normalized to the maximum peak K_v1.3 in the presence of 10 μM Glu for reasons of comparison. E, average g-V plots in 10 μM (▼), 100 μM (■), and 1000 μM (★) Glu normalized to 10 μM Glu (E1) or to the maximum conductance of each treatment (E2). The midpoint of activation is shifted back toward Glu-free values (-31.3 ± 1.2) to -34.3 ± 1.4 mV and -32.1 ± 1.7 mV in 100 and 1000 μM Glu, respectively, compared with -42.6 ± 1.7 mV at 10 μM Glu. The voltage dependence of the channel is markedly reduced as seen by the decrease in the steepness of the g-V curve.

(Fig. 3B). Activation and deactivation rates were similar at all Glu concentrations (Fig. 3C2). However, a faster and more pronounced current decay (fractional current reduction at the end of the pulse) was evident as the concentration of Glu increased, reflecting a faster current inactivation. The increased rate of inactivation in the presence of Glu (Fig. 3C3) was not accompanied by changes in cumulative inactivation (Fig. 3C4) or in time of recovery from inactivation. Furthermore, the inactivation rate of the current in both Glu (10 to 1000 μM) and Glu-free bath solution decreased as the extracellular K^+ concentration increased from 5 to 140 mM, consistent with C-type inactivation of the current. When the bath contained 140 mM K^+, Glu minimally enhanced current inactivation. The above data suggest that Glu does not stabilize the channel into its inactivated state and that the faster current decay is most probably caused by enhancement of C-type inactivation. The dose-dependent increase on inactivation suggests that increasing concentrations of Glu favor the transition of the open to the inactivated (nonconducting) state of the channel. Thus, the total IK_v in response to depolarizing stimuli will be decreased in the presence of elevated concentrations of Glu, because there will be fewer channels conducting at any one time at any given potential. This is clearly shown in Fig. 3, D1 and D2 (compare with Fig. 1C3), where we present I_{Kv} whole-cell responses in the presence of 100 and 1000 μM Glu (pathological plasma range), normalized to the maximum current at 10 μM Glu (normal plasma level). The rate of current activation did not differ significantly compared with 10 μM Glu (Fig. 2A), whereas the inactivation rate increased (or the τ_j value decreased) in a statistically significant manner ($p < 0.005$) as the extracellular concentration of Glu increased, at all voltages tested (Fig. 2B). At -20 mV, τ_j values were 116 ± 6 ms ($n = 18$) for 100 μM and 99 ± 8 ms ($n = 20$) for 1000 μM Glu, respectively (compared with 130 ± 8.2 ms at 10 μM Glu).

Activation curves (g-V) from peak IKv normalized to the maximum conductance at 10 μM ($g_{re}l$; Fig. 3E1) exhibited a marked decrease in the K^+ conductance at elevated Glu concentrations. Moreover, the midpoint of activation, from activation curves normalized to the maximum conductance at each treatment (g_{norm}; Fig. 3E2), was shifted toward more positive potentials by 8.3 ± 2.2 ($n = 23$) and 10.5 ± 2.4 mV ($n = 25$) at 100 and 1000 μM, respectively, compared with 10 μM Glu ($n = 20$). The slope (k_n) values of the g-V curves were 13.8 ± 2.5 and 14.9 ± 2.2 at 100 and 1000 μM, respectively, versus 9.6 ± 1.5 at 10 μM Glu. This decrease in the voltage dependence of the $K_v1.3$ conductance at elevated Glu concentrations indicates that the channel will be less responsive to changes in the membrane potential. To further quantify the effect of the various Glu concentrations on the total K^+ efflux, we calculated the charge quantity (Q_K) conducted through $K_v1.3$ channels during a depolarizing stimulus to -20 mV. As depicted in Fig. 4, elevated Glu concentrations induce a dramatic reduction of the total potassium efflux, which, at 1000 μM Glu, reaches only $26 \pm 9\%$ of the efflux observed at normal (10 μM) plasma concentrations.

Effects of Extracellular Glu on Steady-State Inactivation of $K_v1.3$ Channels. Steady-state inactivation provides information about the number of channels available to activate when the T-lymphocyte membrane has remained to a specific potential for an extended time period. Hence, it is particularly informative of the availability of active channels around the resting potential, where the cells spent most of their time. Using appropriately designed voltage protocols (see *Materials and Methods*), we tested the effects of Glu (1–1000 μM) on the steady-state inactivation of Kv channels. Data from these experiments (Fig. $5A_1$) revealed that, in Glu-free solution ($n = 35$), the IK_v amplitude in response to a depolarizing step to +40 mV (test-pulse) decreases significantly (inactivates) after a prolonged stay (100 s) of the membrane to a potential of -60 mV (prepulse). The amplitude of the current elicited at the test-pulse depends on the fraction of channels available to activate at a given prepulse potential. In the presence of Glu, IK_v starts to inactivate at a more negative prepulse potential (-75 mV, $n = 40$), with the degree of inactivation being more pronounced as the Glu concentration increases (Fig. 5, A2 and A3). Inactivation current-prepulse potential relations in Fig. 5B reveal a leftward shift of the voltage dependence of steady-state inacti-

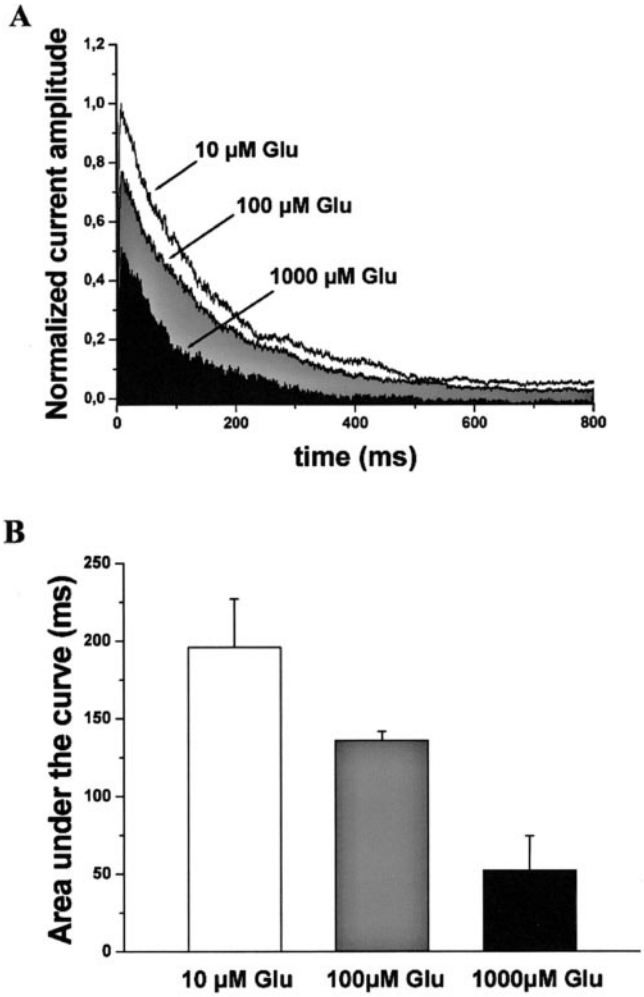

Fig. 4. Effects of increasing Glu concentrations on the total K^+ flux carried by Kv channels. A, integrating current traces elicited during a 800 ms depolarizing voltage step to -20 mV yields the K^+ charge quantity conducted by $K_v1.3$ channels in the presence of 10, 100, and 1000 μM Glu. Currents were recorded after perfusion with the corresponding Glu concentration for 3 min. B, the charge quantity (Q_K) shows a marked reduction as the Glu concentration increases above physiological (10 μM) levels. To overcome cell-to-cell variability, Q_K calculated under these concentrations was normalized to the value obtained at 10 μM for each cell. In the presence of 100 and 1000 μM Glu, Q_K is reduced by 31 ± 3 and $74 \pm 9\%$, respectively, compared with the total charge conducted at 10 μM Glu. Averages were obtained from a total of seven cells.

vation in the presence of 10, 100, and 1000 μM Glu. The midpoint of current inactivation was shifted, compared with Glu-free solution, by -13.2 ± 2.1 mV ($n = 22$; $p < 0.001$), -16.3 ± 2.3 mV ($n = 17$; $p < 0.001$), and -17.2 ± 1.9 mV ($n = 20$; $p < 0.001$) in 10, 100, and 1000 μM Glu, respectively (Fig. 5B). A significant difference ($p < 0.005$) exists only between 10 and 100 or 1000 μM Glu, whereas p values between 100 and 1000 μM or between 1 and 10 μM Glu were not significant.

Metabotropic and Non-NMDA Ionotropic Glutamate Receptors Are Involved in K_v1.3 Modulation. In the CNS, Glu is known to either directly mediate or modulate neuronal excitation through ionotropic or metabotropic receptor activation. In an effort to clarify whether IK_v modulation by Glu in T lymphocytes is also exerted through activation of cognate receptors, we investigated the effects of agonists able to activate either the ionotropic [kainate (10 and 100 μM) and NMDA (5 and 10 μM)] or the metabotropic [*trans*-(1*S*,3*R*)-ACDP and DCG-IV] Glu receptors.

The expression of functional ionotropic non-NMDA Glu receptors was recently shown in T lymphocytes (Ganor et al., 2003). Application of 5 or 10 μM NMDA under our experimental conditions had no significant effect on K_v1.3 properties (data not shown). In contrast, addition of kainate (10 and 100 μM), an agonist for the non-NMDA type of ionotropic Glu receptors (Borges et al., 1994), caused a dose-dependent suppression of current responses. Figure 6A1 shows that, in the presence of 10 μM kainate, currents were reduced in amplitude and inactivated twice as fast compared with control ($\tau_j = 98 \pm 11$ ms, $n = 7$, versus 168.1 ± 9.6 ms, $n = 20$). The agonist had no effect on the rate or threshold of current activation (compare Figs. 1C1 and 6A1), whereas it increased cumulative inactivation and time of recovery from inactivation, suggesting that this agonist stabilizes the active channel in its inactivated state. The decrease in current amplitude in most cells was more pronounced at voltages more positive to -35 mV. Kainate caused a shift in the g-V activation curve to more positive potentials by approximately 4 and 8 mV at 10 and 100 μM, respectively, as estimated by the shift of the midpoint of activation (Fig. 6A2) compared with control. Kainate had no effect on the steady-state inactivation (Fig. 6A3). These effects of kainate on K_v1.3 are similar to those reported for the delayed rectifier K_v channels in oligodendrocytes (Borges et al., 1994; Gallo et al., 1996). In these cells, the enhancement of K_v current inactivation and the subsequent current decrease was caused by Na^+ influx through the non-NMDA channel upon receptor activation (Gallo V. et al., 1996). In our recordings, however, we detected no large changes in the current noise to justify non-NMDA channel activation by kainate, as is the case in glia or neuronal cells (Borges et al., 1994; Poulopoulou and Nowak, 1998). Nevertheless, this does not rule out the possibility that such channels are present in T lymphocytes, because if their number is low and their conductance small, we would be unable to detect them. Further studies are required to elucidate the effects of non-NMDA receptor activation on the K_v1.3 channel of T lymphocytes. The above data, however, show that kainate alters the K_v1.3 current through a different mechanism than the one of Glu. Further support for that comes from experiments in which Glu was coapplied in the bath with CNQX, a non-NMDA receptor antagonist. Presence of CNQX in the bath had no measurable effect on the

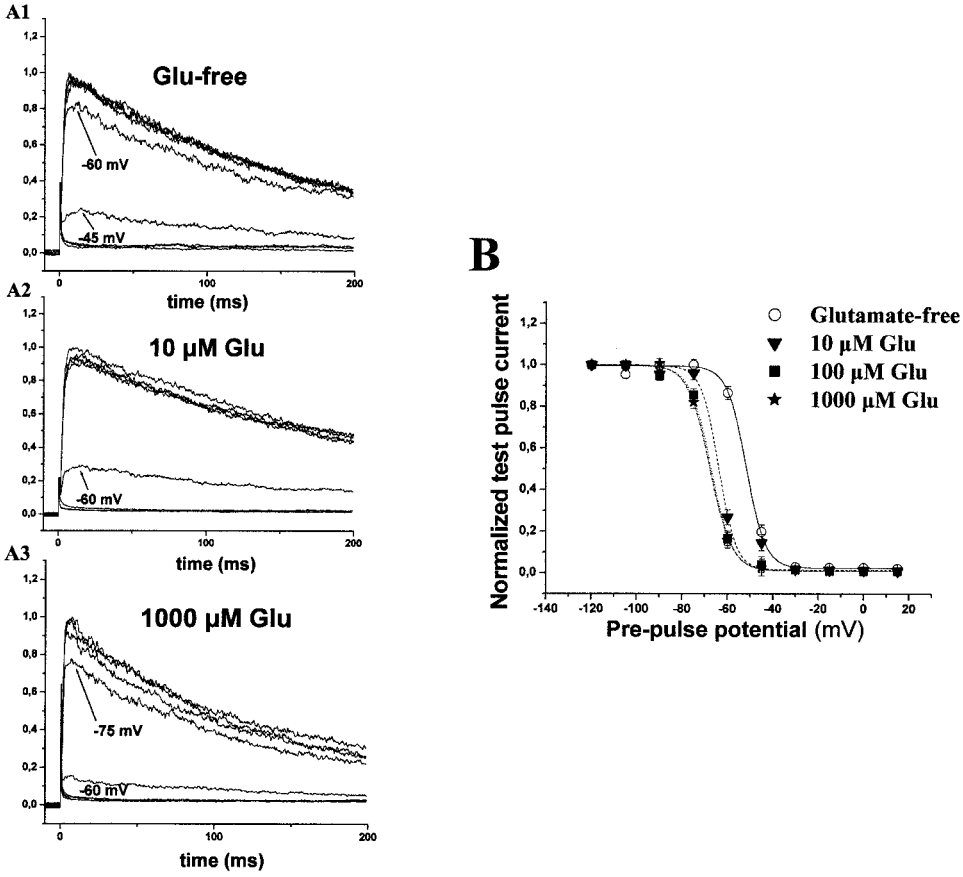

Fig. 5. Glutamate affects the voltage dependence of steady-state inactivation. A, a T-lymphocyte held at -90 mV and stepped every 70 s to a conditioning prepulse (-120 to $+30$ mV) for 100 s, followed by a 200-ms test pulse to $+40$ mV in Glu-free bath (A1) and then sequentially exposed to 10 (A2) and 1000 μM Glu (A3). The current elicited in response to the test pulse defines the fraction of channels available to activate after each prepulse potential. This fraction appears as the normalized peak current relative to the current evoked at -120 mV for each treatment and is plotted against the prepulse potential (millivolts). B, inactivation curves in the presence of 10 μM (▼), 100 μM (■), and 1000 μM (★) Glu are shifted to the left compared with Glu-free (○), and the midpoint of inactivation is -51.5 ± 1.4 mV for Glu-free, -64.7 ± 1.6 for 1 and 10 μM Glu, and -67.8 ± 1.8 mV for 100 and 1000 μM Glu.

Glu effects on IK_v. Again, this does not exclude the possibility of a kainate receptor contribution to the effects of high concentrations of Glu, because CNQX-insensitive low conductance kainate currents have been previously reported (Poulopoulou, 1994).

The next step was to investigate whether metabotropic Glu receptor agonists could mimic the effects of Glu on the $K_v1.3$ channel. The nonselective metabotropic Glu-receptor agonist *trans*-ACPD (Schoepp et al., 1999), at 100 μM, completely mimicked the effects of 1 μM Glu on Kv currents (compare Figs. 6B1 and 1C2). In the presence of this agonist, currents started to activate at -50 mV with a faster activation (τ_n = 1.8 ± 0.1 ms, -20 mV) and inactivation (τ_j = 159 ± 9 ms, -20 mV) rate, whereas both steady-state activation and inactivation were shifted to more negative potentials by -11.35 ± 1.7 mV and -9.76 ± 2.5 mV, respectively (Fig. 6, B2 and B3). These data show that the effects of 1 and 10 μM Glu may be brought on by the activation of metabotropic receptor(s), suggesting the expression of functional metabotropic receptors with high affinity for Glu on human T lymphocytes. This is in accordance with binding data, in which it was shown that the high-affinity Glu binding site is a metabotropic site with a K_d for Glu of 0.3 μM (Kostanyan et al., 1997). Current recordings in the presence of specific group I, II, and III metabotropic Glu receptor agonists suggest that the effects of Glu on the $K_v1.3$ channel are exerted through activation of group I and II metabotropic receptors, whereas the group III agonist [L(+)-2-amino-4-phosphonobutyric acid] had no effect on the current.

$K_v1.3$ currents in the presence of a 10 μM concentration of the group I selective metabotropic receptor agonist (*S*)-DHPG (n = 8; data not shown) in the bath were enhanced in

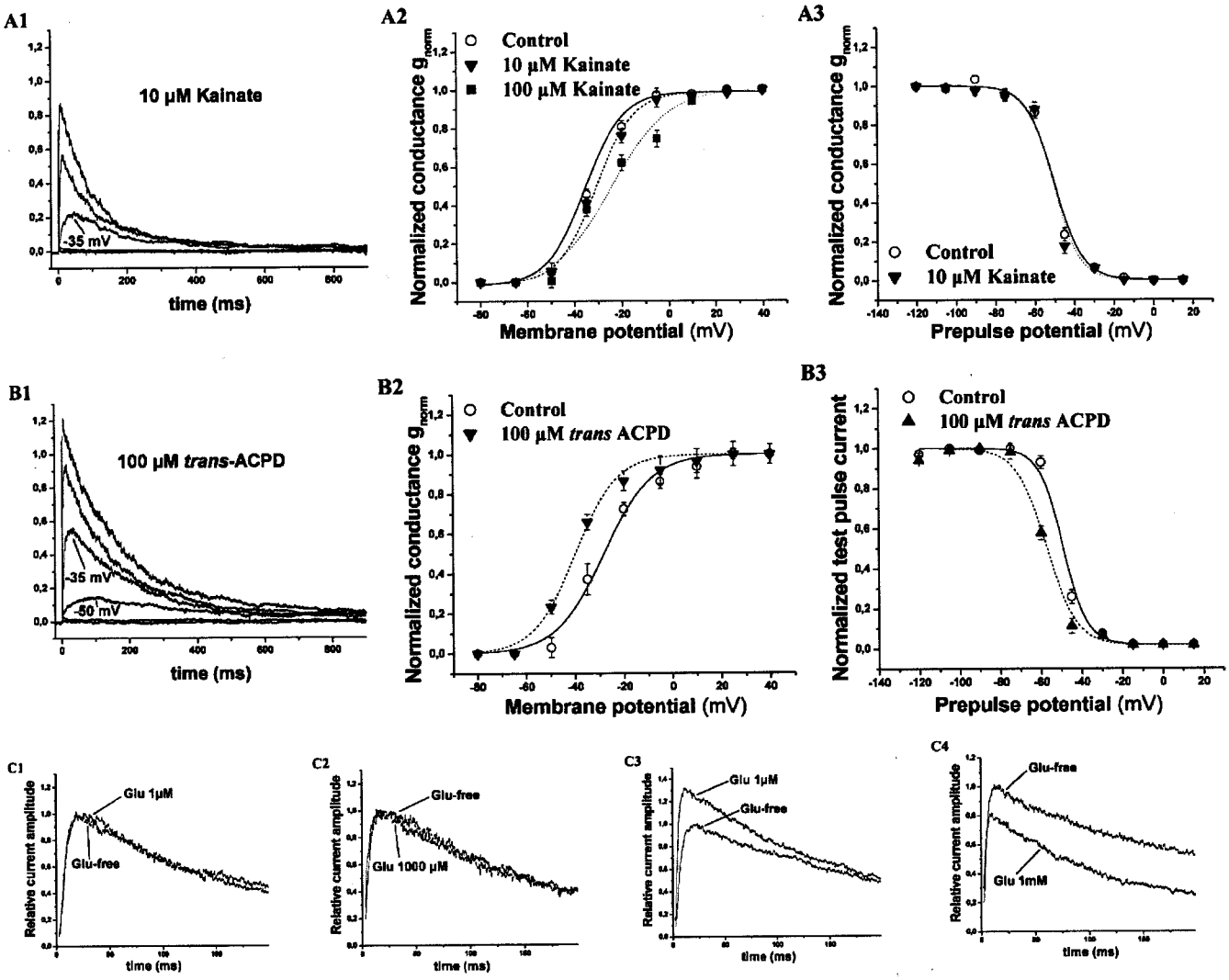

Fig. 6. T lymphocytes express functional metabotropic and non-NMDA ionotropic Glu receptors. Currents are presented relative to the maximum peak current amplitude of the agonist-free control. A, I_{Kv} responses from a T lymphocyte bathed in 10 μM kainate inactivate at a significantly faster rate and are reduced in amplitude. Currents are normalized to the maximum I_{Kv} agonist-free solution (A1). Steady-state activation (A2) and inactivation (A3) conductance-to-voltage plots of agonist-free (control) (○), 10 μM (▼), and 100 μM (■) kainate. B, I_{Kv} responses in the presence of a 100 μM concentration of the metabotropic Glu receptor agonist *trans*-ACPD (B1). Activation (B2) and inactivation (B3) g-V plots are both shifted to the left by *trans*-ACPD (▲) compared with control (○). C, effects of guanosine nucleotide analogs on the action of 1 and 1000 μM Glu. Inclusion of the nonhydrolyzable analog GDPβS (1000 μM) in the pipette solution totally blocked the action of 1 (C1) or 1000 μM (C2) Glu on $K_v1.3$ currents. In contrast, inclusion of the nonhydrolyzable analog GTPγS (250 μM) in the internal solution led to maximal current potentiation after application of 1 μM Glu (C3). Under these experimental conditions, addition of 1000 μM Glu markedly suppressed $K_v1.3$ currents, although the rate of inactivation was not consistently affected (C4). Current traces are from repetitive step depolarizations to -20 mV.

amplitude and activated at −50 mV with faster activation (τ_n = 1.7 ± 0.2 ms, −20 mV) and inactivation (τ_j = 147 ± 7 ms, −20 mV) rates. The midpoint of activation and steady-state inactivation were both shifted toward more hyperpolarized potentials by about −12 and −10 mV, respectively, thereby increasing the number of active channels at more negative voltages. On the other hand, application of the group II metabotropic receptor agonist DCG-IV at 10 μM (n = 6; data not shown) reduced IK_v amplitude with no effect on the activation threshold potential or current kinetics (activation or inactivation rates), whereas it shifted the steady-state inactivation to more negative potentials by about −8 mV. This suggests that the current reduction by DCG IV is caused by the increase in steady-state inactivation.

Additional support for the action of Glu on the K_v1.3 channel through metabotropic Glu receptors comes from experiments in which the nonhydrolyzable analogs of GDP (GDPβS) and GTP (GTPγS) were included in the intracellular solution (pipette). In general, the first step after metabotropic receptor activation, including the metabotropic glutamate receptor, is the activation of a G-protein, although in some cases G-protein-independent effects of metabotropic glutamate receptors have also been reported (Heuss et al., 1999). G-protein activation requires the exchange of bound GDP (resting state) with GTP (activated state). This is a common and necessary step of all G-protein mediated actions and is independent of G-protein type or the second messenger system involved. Therefore, blockade of this exchange will result in inability of the ligand-bound receptor to exert its action, whereas acceleration of this substitution will enhance its effects. Application of 1 and 1000 μM Glu in the bath exerted negligible effects on the electrical properties of the K_v1.3 current, when 1 mM GDPβS was included in the pipette (Fig. 6, C1 and C2). To the contrary, when the intracellular solution contained 250 μM GTPγS, the effects of Glu at both 1 and 1000 μM were enhanced (Figs. 6, C3 and C4). Therefore, activation of group II metabotropic receptors will decrease the voltage range at which K_v1.3 channels are active.

Our findings strongly indicate that K_v1.3 modulation by Glu involves receptor-mediated pathways. However, we cannot completely rule out the possibility that Glu has additional direct actions on K_v1.3 channels. Further studies in recombinant cells expressing cloned K_v1.3, are needed to resolve this issue.

Discussion

The density and activity of delayed rectifier potassium channels is known to correlate with the differentiation and proliferation of cell types as diverse as T lymphocytes (DeCoursey et al., 1984) and Schwann cells (Sobko et al., 1998). These fast-activating and slowly inactivating channels have an integrative role in the physiology of T lymphocytes that includes the regulation of membrane potential (Cahalan et al., 2001), calcium signaling (Lin et al., 1993), lymphokine secretion (Chandy et al., 1984), integrin function (Levite et al., 2000), and mitogen-stimulated proliferation (DeCoursey et al., 1984). Therefore, any modulation of the electrical properties of these channels is expected to have a direct effect on T-lymphocyte physiology. The present report provides direct evidence that the endogenous ligand Glu differentially modulates native K_v1.3 channels of human T lymphocytes, in a concentration-dependent manner, and postulates on the mechanism through which Glu exerts its action on T-lymphocyte function. The modulation of K_v1.3 by Glu has biological significance because the ligand is effective at concentration ranges within those found in the extracellular space of tissues (such as blood, CNS, liver, etc.) under both normal and pathological conditions.

One of our key findings was that low and elevated concentrations of Glu affect IKv amplitude in a strikingly different manner: whereas 1 μM Glu maximally enhances IKv, at high concentrations (≥100 μM), its role becomes inhibitory and causes a dramatic current reduction. It is interesting that an array of in vitro data shows that low and elevated Glu concentrations can differently affect important cellular events such as stimulated Ca^{2+} signal (Lombardi et al., 2001), cell cycle progression (Sommer et al., 1994), and integrin-mediated adhesion (Ganor et al., 2003). In fact, maximal intracellular Ca^{2+} elevation is reported in response to mitogenic stimulation at 1 μM Glu, whereas, at 100 μM and above, this effect is hindered and proliferation is suppressed. Given that the activity of K_v channels regulates the degree of Ca^{2+} influx and thus Ca^{2+} signal and Ca^{2+}-dependent processes (Hess et al., 1993; Lin et al., 1993; Verheugen and Vijverberg, 1995; Verheugen et al., 1997), our results on modulation of the K_v channel by Glu provide a plausible mechanism for the distinct effects of Glu on T lymphocytes.

The dose-dependent differences in the effects of Glu on IK_v rise from the integration of distinct alterations in the electrical properties of the K_v1.3 channel. Glu at 1 μM highly promotes the transition of the K_v1. 3 channel to its open state, at potentials more positive than −50 mV, without affecting current inactivation or deactivation. This leads to an increase in the number of active conducting versus nonconducting channels (increased whole-cell conductance), which in turn translates into maximal membrane hyperpolarizations in response to a depolarizing immune stimulus. This potentiates the driving force for Ca^{2+} entry through CRAC channels and thus may account for the maximal elevation of stimulated intracellular Ca^{2+} reported (Lombardi et al., 2001) at this concentration. At 10 μM Glu, currents show a small but statistically significant reduction in amplitude, compared with 1 μM, whereas they remain enhanced compared with the Glu-free solution for potentials physiologically relevant to nonexcitable cells (−50 to −5 mV). At 10 μM Glu, IK_v activates at the same rate but inactivates faster, which accounts for this decrease. Therefore, at this concentration, K^+ efflux becomes smaller than at 1 μM Glu but is still large enough to counteract depolarizations and keep the necessary negative membrane potential for Ca^{2+} influx. As mentioned above, elevation of Glu concentration to ≥10 μM brings forth a dose-dependent increase in the rate of current inactivation that leads to a gradual decrease in the number of conducting channels during a depolarization. At the same time, Glu at concentrations of ≥100 μM increases resting (i.e., closed-state) channel inactivation, thereby rendering a greater number of channels unavailable to open in response to a stimulus. The combined increase on steady-state and current inactivation can account for the dramatic decrease of IK_v in the presence of elevated Glu concentrations. The observed reduction in total K^+ efflux by 70% at 1000 μM Glu may explain the decrease in Ca^{2+} signal and proliferation

reported at this concentration (Eck et al., 1989; Sommer et al., 1994; Lombardi et al., 2001). IKv reduction of similar magnitude has been reported to decrease IL-2 and IL-4 production (Freedman et al., 1992; Ghanshani et al., 2000), and proliferation (Gaspar et al., 1996; Ehring et al., 1998; Conforti et al., 2003).

It is interesting to note that, even at elevated Glu concentrations, currents still activate at −50 mV in contrast to the Glu-free solution. Thereby, in the wide concentration range of 1 to 1000 μM, Glu promotes the responsiveness of Kv channels at voltages near the T-lymphocyte resting membrane potential (−50 to −60 mV) (Cahalan et al., 2001). This negative shift in the activation threshold is of major importance for T lymphocytes, because most immune stimuli will cause small extrusions from the resting potential. In fact, it may explain the minimal intracellular Ca^{2+} elevation and the absence of proliferation when T lymphocytes are stimulated in the absence of extracellular Glu (Lombardi et al., 2001).

Based on the differential modulation of $K_v1.3$ by low and high glutamate concentrations, we suggest that in vivo the responsiveness of T lymphocytes in different tissues will be determined at least in part by their extracellular content of Glu. One may thus predict that, at normal plasma levels of Glu (10 to 50 μM), T lymphocytes will have a better ability to keep a negative membrane potential and thus an optimal response to immune stimuli. It is interesting that at elevated plasma Glu, T lymphocytes are expected to have impaired functions because of the significantly decreased $K_v1.3$ channel activity. This is in accordance with clinical studies reporting a strong correlation between elevated plasma Glu and immunosuppression. Finally, in the CNS, where extracellular Glu concentration is approximately 3 μM (Danbolt, 2001), T lymphocytes are more likely to activate than when they circulate in the peripheral blood.

The functional consequences of $K_v1.3$ modulation are expected to be more prominent on T lymphocytes of the effector memory (T_{EM}) subset. This is justified by the functional dominance of $K_v1.3$ channels throughout the activation of these cells, in contrast to naive and central memory (T_{CM}) cells, where $K_v1.3$ dominance is limited to the initial steps of activation (Wulff et al., 2003; Chandy et al., 2004). Thus one may speculate that elevated plasma Glu will selectively impair the memory response (which depends on T_{EM} cells) as to the bulk of cellular immunity.

Possible Mechanisms of $K_v1.3$ Modulation. Different molecules exert their effects on $K_v1.3$ current amplitude or kinetics, as a consequence of their physical interaction with the channel pore or gates (Cahalan et al., 1985; Bregestovski et al., 1986; Deutsch and Lee, 1989). A similar effect of Glu cannot be eliminated by our data, imposing the need for future experiments on $K_v1.3$ channels expressed in a recombinant system. However, based on data showing that: 1) the channel is not stabilized into any of its active states (conducting or nonconducting), 2) current inactivation in the presence of Glu follows C-type properties (e.g., slow-down in the presence of high extracellular K^+), and 3) inclusion of GDPβS in the recording pipette alleviates the effects of glutamate on the current, we propose that the alterations of $K_v1.3$ properties induced by Glu involve chemical modifications of the channel protein.

The complex effects of Glu on $K_v1.3$ may result from the activation of distinct Glu receptor types with different affinities for the ligand. T lymphocytes have been found to express Glu receptors of both the ionotropic (Lombardi et al., 2001; Ganor et al., 2003) and the metabotropic (Storto et al., 2000; Pacheso et al., 2004) subtypes. Functional non-NMDA ionotropic receptors are present on human T lymphocytes and their agonist kainate is able to mimic the effects of nanomolar Glu concentrations on integrin-mediated T lymphocyte adhesion (Ganor et al., 2003). However, this effect of kainate probably regards a separate action of the non-NMDA receptor-channels, because we found that this agonist decreases $K_v1.3$ currents by stabilizing active channels into their inactivated state. A similar action of kainate has been reported for the delayed rectifier K^+ current of oligodendrocyte progenitor cells (Borges et al., 1994; Gallo et al., 1996), and it was shown to be secondary to kainate receptor-channel activation, because the K^+ current was blocked by the increase in intracellular Na^+ (Borges and Kettenmann, 1995).

On the other hand, the data from the metabotropic Glu receptor agonists argue that Glu acts on $K_v1.3$ channels through metabotropic receptor activation (Table 1). Group I metabotropic agonist (*S*)-DHPG at a concentration of 10 μM potentiated $K_v1.3$ responses similarly to low Glu concentrations (1 and 10 μM), suggesting a high affinity of group I receptors for the endogenous ligand. On the other hand, the group II metabotropic Glu receptor agonist DCG-IV decreased the maximum conductance of the channel by increasing resting (steady-state) channel inactivation, an effect seen only at 100 μM Glu and above, suggesting a lower affinity of these receptors for Glu.

Based on the above and on the finding that inclusion of GDPβS in the pipette solution alleviates the effects of Glu (1

TABLE 1

Summary of the effects of Glu and its agonists on the K_v 1.3 currents

K_v 1.3 currents in Glu-free solution have a threshold potential of −35 mV, they activate fast (τ_n =3.5 ± 0.5 ms) and inactivate slowly (τ_j =168.1 ± 9.6 ms), the voltage of their half maximal activation ($V_{1/2}$ act) is approximately −31 mV, whereas half of these channels have entered their inactive state ($V_{1/2}$ inc) at approximately −52 mV. In this table, we present the effects of Glu (1 to 1000 μM) and its agonists on the basic K_v 1.3 properties. All comparisons are with Glu-free solution.

Agonist and Concentration	Glu				KA 100 μM	ACPD 100 μM	DHPG 10 μM	DCG-IV 10 μM
	1 μM	10 μM	100 μM	1000 μM				
IK_v 1.3 properties								
Activ. threshold (mV)	−50	−50	−50	−50	−35	−50	−50	−35
G_{max}	↑++	↑+	↓+	↓++	↓+++	↑+	↑+	↓++
τ_n	↑++	↑++	↑++	↑++	—	↑++	↑++	↑++
τ_j	—	↑+	↑++	↑+++	↑++++	↑+	↑+	—
$V_{1/2}$ act (mV)	−45	−43	−34	−32	−23	−43	−44	−30
$V_{1/2}$ inc (mV)	−65	−65	−68	−68	—	−61	−62	−62

G_{max}, maximum whole-cell conductance; τ_n, activation time constant; τ_j, inactivation time constant; ↑, increase; ↓, decrease; —, unchanged; +, ++, intensity of change.

to 1000 μM), one might postulate that Glu would modulate IK_v mainly through the activation of metabotropic receptors. Low Glu concentrations probably act via group I metabotropic receptors, whereas the effects of higher doses seem to result from the combined activation of both group I and II receptors. Our data cannot exclude that ionotropic Glu receptors contribute to the effects seen at high Glu concentrations, but this contribution must be a small one, because coapplication of Glu with CNQX (a non-NMDA antagonist) cannot alleviate the effects at a detectable level (data not shown). Further studies are needed to discriminate between the separate effects of Glu and the intracellular pathways involved.

In conclusion, the present study demonstrates a biologically significant modulation of native $K_v1.3$ channels of human T lymphocytes by an endogenous ligand-receptor interaction. This modulation is intricate enough so that this ligand (Glu) may be able to finely regulate T lymphocyte responses, depending on its concentration and on the receptor types expressed on their membrane. Therefore, Glu may be regarded as a key regulatory factor in the immune system; although it is essential for proper T lymphocyte activation and proliferation, it may act as an immunosuppressant when its plasma concentration rises to pathological levels, as is the case for AIDS and cancer. Apart from their obvious physiological importance, our findings also provide a target mechanism for future pharmacological manipulations in therapeutic strategies against immune disorders.

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