British Journal of Pharmacology (1999) 128, 1235–1240
http://www.stockton-press.co.uk/bjp

Pharmacological activation of the ryanodine receptor in Jurkat T-lymphocytes

*,[1]Martin Hohenegger, [2]Ingeborg Berg, [3]Lukas Weigl , [2]Georg W. Mayr, [4]Barry V.L. Potter & [2]Andreas H. Guse

[1]Pharmacological Institute, University of Vienna, Waehringerstr.13a, A-1090 Vienna, Austria; [2]Institute of Physiological Chemistry, Department of Enzyme Chemistry, University of Hamburg, Grindelallee 117, D-20146 Hamburg, Germany; [3]General Hospital of the University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria and [4]Wolfson Laboratory for Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Claverton, Bath BA2 7AY

1 Recently, we provided evidence for cyclic adenosine 5′-diphosphate-ribose, cADP-ribose, as a second messenger in Jurkat T-lymphocytes upon stimulation of the T-cell receptor/CD3- complex (Guse *et al*., 1999). cADP-ribose mobilizes Ca^{2+} from an intracellular Ca^{2+} store which is sensitive to caffeine and gated by the ryanodine receptor/Ca^{2+} release channel. In the present study we investigated the ability of the trypanocidal drug, suramin, to activate the ryanodine receptor of T-cells. Since suramin cannot permeate the plasma membrane, it was necessary to microinject the drug into Fura-2 loaded T-lymphocytes.

2 In a dose dependent manner suramin increased the intracellular Ca^{2+} concentration. The dose-response curve is very steep and calculates for an EC_{50} of 7.6 ± 2.9 mM suramin in the injection pipette.

3 Co-injection of the selective ryanodine receptor inhibitor ruthenium red completely abolished the suramin induced Ca^{2+} transient. This finding allows for the conclusion that the IP_3-receptor sensitive Ca^{2+} pool is not the primary target of the suramin induced Ca^{2+} transient.

4 Furthermore, Ins(1,4,6)PS_3, an antagonist of the $InsP_3$-receptor could not suppress the suramin-induced Ca^{2+} signal. The suramin induced Ca^{2+} transients declined very slowly; however, in the presence of Ins(1,4,6)PS_3 this decay was accelerated. In addition, suramin did not interact with the cADP-ribose binding site of the ryanodine receptor of T-cells.

5 In conclusion, suramin is found to be an agonist for the T-cell ryanodine receptor as previously found for the cardiac and skeletal muscle isoform. Therefore, suramin can be designated a universal ryanodine receptor agonist.

Keywords: Jurkat T-lymphocytes; ryanodine receptor; suramin; cyclic ADP-ribose; inositol triphosphate; T-cell activation

Abbreviations: cADP-ribose, cyclic adenosine 5′-diphosphate ribose; Ins(1,4,6)PS_3, D-myo-inositol 1,4,6-trisphosphorothioate; 8-OCH_3-cADP-ribose, 8-methoxy cyclic adenosine 5′-diphosphate ribose; RyR, ryanodine receptor

Introduction

The cytosolic Ca^{2+} concentration is essentially linked with the immunoreactivity of T-lymphocytes (Guse, 1998). Activation of the T cell receptor/CD3-complex results in elevated cytosolic Ca^{2+} concentrations. In this process participation of the $InsP_3$-dependent Ca^{2+} pool has been described extensively (Jayaraman *et al*., 1995; for review see Crabtree & Clipstone, 1994). Recently, we have demonstrated that activation of the T-cell receptor/CD3-complex is accompanied by a sustained generation of the novel second messenger, cyclic adenosine 5′-diphosphate ribose, cADP-ribose, which is observed in parallel to sustained Ca^{2+} signalling (Guse *et al*., 1999). The molecular target for the cADP-ribose induced Ca^{2+} signals was identified as the ryanodine receptor (RyR).

The RyR like the $InsP_3$-receptor, is a member of the family of intracellular Ca^{2+} release channels. The RyR plays an essential role in the excitation-contraction coupling of the skeletal and cardiac muscle (Marks, 1997). The ion channel consists of a tetramer of four identical $\sim$560 kDa subunits. The physiological regulation of the skeletal (type 1) and cardiac (type 2) isoform of the RyR involves gating by the plasma membrane dihydropyridine receptor *via* an electro-mechanical coupling or a Ca^{2+} influx, respectively (Clapham, 1995). Both isoforms of the RyR seem to be restricted to excitable cells; however, a third isoform (type 3) was cloned and termed the brain isoform (Hakamata *et al*., 1992; Nakashima *et al*., 1997). Although the RyR type 3 is abundant in some brain areas it is also found in non-excitable cells like spleen and testis (Giannini *et al*., 1995). Due to the low expression level of type 3 RyR little is known about the physiological and pharmacological regulation of this RyR isoform. Most of the information of the type 3 ion channel is derived from the primary sequence. The distribution of the putative transmembrane domains of the RyR type 1 and 2 are also found in the RyR type 3. A nucleotide binding site and three potential calmodulin binding sites are predicted (Leeb & Brenig, 1998).

In Jurkat T-lymphocytes a RyR was identified by Western blot and [^{3}H]-ryanodine binding assays. RT–PCR with specific primers for a COOH-terminal fragment indicated that the RyR in Jurkat T-lymphocytes is the type 3 RyR, while RyR's type 1 and type 2 were absent (Guse *et al*., 1999). This finding is corroborated by the sensitivity of T-lymphocytes for cADP-ribose. The RyR's type 1 and type 2 have been found to be insensitive toward exposure to cADP-ribose (Fruen *et al*., 1994). The sole presence of the type 3 RyR makes the Jurkat T-lymphocyte a prototypical model system to investigate the

*Author for correspondence;
E-mail: martin.hohenegger@univie.ac.at

physiological and pharmacological regulation of this novel RyR isoform.

The trypanocidal drug, suramin, serves as a potent agonist on the RyR isoform type 1 and type 2 (Hohenegger *et al*., 1996). The negatively charged naphthylsulfonyl residues turned out to be essential for the agonistic effects observed for suramin. Otherwise than postulated, the interaction of suramin does not take place *via* an ATP binding site of the ryanodine receptor (Emmick *et al*., 1994; Hohenegger *et al*., 1996). Calmodulin, a negatively charged Ca^{2+} binding protein is a dualistic regulator of the RyR (Meissner, 1994). Very recently, we could show that suramin competes with calmodulin for the binding to type 1 RyR (Klinger *et al*., 1999). The fact that the type 3 RyR conserved three predictive calmodulin binding sites led us to the question whether this isoform of the ryanodine receptor is sensitive toward suramin or not.

In the present study we have loaded Jurkat T-lymphocytes with Fura 2 and measured the fluorescence changes due to the addition of suramin. We circumvented the impermeability of the plasma membrane for suramin by usage of the microinjection technique. Upon suramin injection the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of T-cells was elevated in a dose-dependent manner. This Ca^{2+} transients were unequivocally mediated *via* a ryanodine sensitive Ca^{2+} pool.

Methods

Materials

8-methoxy cyclic adenosine 5′-diphosphate ribose, (8-OCH_3-cADP-ribose), was synthesized briefly as follows: 8-bromo-AMP was treated with sodium methoxide to convert it into 8-methoxy AMP which was purified by ion-exchange chromatography and subsequently coupled to nicotinamide adenine mononucleotide using dicyclohexyl carbodiimide by a method analogous to that previously reported (Ashamu *et al*., 1997). The resulting 8-methoxy NAD was cyclized to 8-methoxy cADP-ribose using *Aplysia* ADP-ribosyl cyclase and was purified by ion-exchange chromatography and thoroughly characterized by HPLC, NMR and mass spectroscopy.

Cyclic ADP-ribose was synthesized from NAD^+ by cyclization with the *Aplysia* ADP-ribosyl cyclase and purified by ion-exchange chromatography on Q-sepharose. Both compounds were used as their triethylammonium salts.

D-myo-inositol 1,4,6-trisphosphorothioate (Ins(1,4,6)PS_3) was synthesized in an analogous fashion to D-Ins(1,4,6)P_3 (Mills & Potter, 1996) except that the intermediate triphosphite was sulphoxidized as described for the racemic material (Mills *et al*., 1995). After purification by ion-exchange chromatography and quantification by total phosphate assay the material was used as its triethylammonium salts.

Digital Ca^{2+} fluorescence measurements and analysis of data

Cultured Jurkat T-lymphocytes were loaded with Fura2-AM and immobilized to thin glass coverslips (0.2 mm) for microinjection as previously described (Guse *et al*., 1993; 1997). The cells were kept in a buffer containing (mM) NaCl 140, KCl 5, $MgS0_4$ 1, $CaCl_2$ 1, HEPES 20, NaH_2P0_4 1, glucose 5.5, pH 7.4. The fluorescence of a single T-lymphocyte was analysed with a digital ratiometric imaging station (PhotoMed GmbH/Photon Technology, Wedel, Germany) using an inverted Axiovert 100 fluorescence microscope (Zeiss, Oberkochen, Germany). The excitation was carried out at 340 nm and 380 nm and the corresponding emission light of 510 nm detected with an CCD camera at a resolution of 525×487 pixel (type C2400-77, Hamamatsu, Garching, Germany) and a data sampling rate of 1 ratio/5 s. Regions of interest covering the investigated injected cell were analysed off line for the 340/380 ratio using the ImageMaster software (PhotoMed GmbH/Photon Technology, Wedel, Germany). The numerical median ratios and the corresponding free Ca^{2+} concentrations were calculated using external calibration.

Microinjection

Microinjection was carried out with an Eppendorf system (transjector type 5246, micromanipulator type 5171; Eppendorf-Netheler-Hinz, Hamburg, Germany) using Femtotips II as pipettes. The compounds were diluted in the intracellular buffer (containing (mM): HEPES 20, KCl 110, $MgCl_2$ 2, KH_2P0_4 5, NaCl 10, pH 7.2) prior to injection to give the concentrations given in the figures and figure legends. The pipette was positioned on top of the cell and then microinjection was initiated by the semiautomatic injection mode of the system. The parameters of the instrumental settings were identical to that described in Guse *et al*. (1997). Under such conditions the injected volume was 1–1.5% of the total cell volume as calibrated by microinjection with the fluorescent dye, Fura 2-free acid.

Miscellaneous procedures

Jurkat T-lymphocytes were cultured in RPMI 1640 medium supplemented with new-born bovine calf serum (10% v v^{-1}), HEPES (25 mM, pH 7.4) penicillin (100 units ml^{-1}), and streptomycin (50 μg ml^{-1}).

The data were subjected to non-linear, least-squares curve fitting using the appropriate equations (rectangular hyperbola, Hill equation and monophasic exponential decay), and parameter estimates were derived.

Results

Application of the trypanocidal drug suramin *via* the microinjection procedure resulted in a tremendous increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in Fura-2 loaded Jurkat T lymphocytes (Figure 1A). The suramin induced increase in $[Ca^{2+}]_i$ was dose-dependent (Figure 1B). Taking the peak-amplitudes of the $[Ca^{2+}]_i$ a curve was fitted according to the Hill equation and calculated for an EC_{50} of 7.6 ± 2.9 mM suramin. This concentration equals that of suramin in the microinjection pipette. Using Fura-2 free acid microinjection under identical conditions and subsequent determination of the dye concentration in the injected cell spectrofluorimetrically, we obtained the dilution factor of a compound using this technique (Guse *et al*., 1997). The final concentration of suramin in the Jurkat T-lymphocyte has to be assumed to be 1–1.5% of that present in the microinjection pipette.

The dose-response relationship for suramin is very steep and calculates for a Hill coefficient of about four. This finding is well in agreement with the previous observation that suramin stimulates open probability of the purified RyR type 1 in single channel recordings with high cooperativity and an EC_{50} of about 200 μM (Hohenegger *et al*., 1996).

The elevated $[Ca^{2+}]_i$ levels were sustained for at least 10 min (Figure 1A). We have therefore determined the t_{600} value, the

time point 10 min after the suramin induced $[Ca^{2+}]_i$ peak concentration (Figure 1C). From these values the calculated EC_{50} of 4.76 ± 1.74 mM suramin was slightly lower than that obtained from the peak $[Ca^{2+}]_i$. The recovery of resting $[Ca^{2+}]_i$ was reduced by increasing concentrations of suramin and significantly different from basal $[Ca^{2+}]_i$. Using the Student's *t*-test we obtained a *P*-value of $P < 0.0005$ and $P = 0.01$ for 10 mM and 20 mM suramin, respectively (Figure 1C).

In order to exclude the possibility that the suramin induced increment in $[Ca^{2+}]_i$ was triggered by targets of the plasma membrane we applied 1 mM suramin to the extracellular bath solution. No alterations in basal $[Ca^{2+}]_i$ were detectable during an observation period of more than 5 min (data not shown). This allows for the assumption that suramin exerted the Ca^{2+} mobilizing activity *via* an intracellular target.

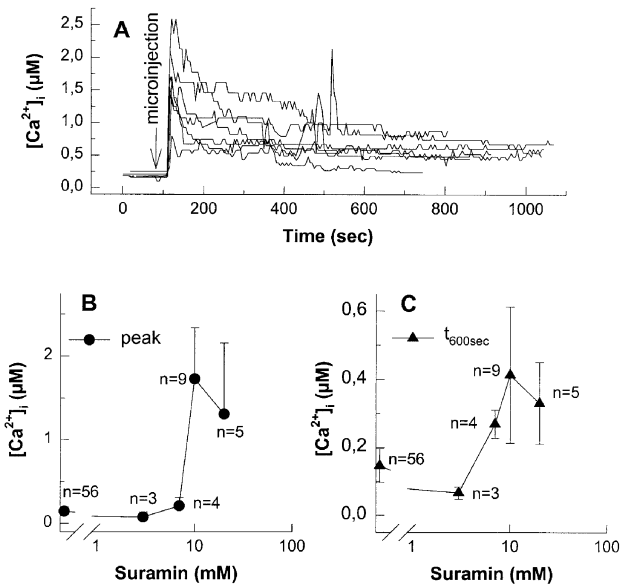

Figure 1 Suramin-induced stimulation of $[Ca^{2+}]_i$. (A) 10 mM Suramin was injected into Fura-2 loaded Jurkat T-lymphocytes and the fluorescence changes were monitored. The ratio of the 510 nm emission signal from excitation at 340 nm and 380 nm was converted into the corresponding free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). $[Ca^{2+}]_i$ from six representative microinjections are shown as overlays. (B) The dose-response curve is given for the peak $[Ca^{2+}]_i$ at increasing concentrations of suramin. (C) The dose-response curve for the time point 600 s after the peak $[Ca^{2+}]_i$ (t_{600}) is given for the indicated suramin concentrations in the injection pipette. The mean of the indicated number of experiments is given for each suramin concentration evaluated for the peak and t_{600} time points. Error bars represent standard deviation.

The primary candidate for suramin binding is the RyR present in Jurkat T-lymphocytes (Guse *et al.*, 1999). The intracellular co-application of a fully stimulating suramin concentration (10 mM) with the RyR specific inhibitor ruthenium red (2 μM) completely abolished the elevation in $[Ca^{2+}]_i$ (data not shown). Even during observation times of more than 20 min no effect on $[Ca^{2+}]_i$ was observed under these conditions. In one out of five cells co-injected with suramin and ruthenium red we detected spontaneous $[Ca^{2+}]_i$ oscillations starting 7 min after the injection time point. Spontaneous $[Ca^{2+}]_i$ oscillations occasionally occur also in the absence of any manipulation in approximately 10% of the investigated cells.

The sustained $[Ca^{2+}]_i$ levels in response to suramin microinjection might be related to the second intracellular Ca^{2+} release channel, the $InsP_3$-receptor (Sugiyama *et al.*, 1994). To prove participation of the $InsP_3$-receptor in Jurkat T-lymphocytes, we used the competitive inhibitor, Ins(1,4,6)PS_3 which we have previously described (Guse *et al.*, 1997). At 80 μM Ins(1,4,6)PS_3, a concentration which completely suppressed the IP_3-mediated activation of the $InsP_3$-receptor (for overview see Table 1), the suramin-induced Ca^{2+} transients were shortened (Figure 2A). The t_{600} values were significantly reduced from 0.53 ± 0.19 μM to 0.26 ± 0.11 μM $[Ca^{2+}]_i$ ($P < 0.005$), in the absence and presence of the $InsP_3$-receptor antagonist, respectively (Figure 2B). However, the peak Ca^{2+} concentrations were not altered by co-injection of Ins(1,4,6)PS_3 (Figure 2B).

In permeabilized T-lymphocytes the presence of ruthenium red only suppressed the cADP-ribose induced $[Ca^{2+}]_i$ elevation but not that induced by $InsP_3$ (summarized in Table 1; Guse *et al.*, 1995). Both compounds, suramin and cADP-ribose, may therefore release Ca^{2+} from the same ryanodine-sensitive Ca^{2+}-pool. Analogous to the antagonist of the endogenous activator of Ca^{2+} mobilization, $InsP_3$, we used a direct antagonist of cADP-ribose, 8-OCH_3-cADP-ribose to possibly identify participation of cADP-ribose in the suramin-induced Ca^{2+}-transient (Figure 3 and Table 1). cADP-ribose is a novel second messenger leading to activation of the RyR upon stimulation of the T-cell receptor. 100 μM 8-OCH_3-cADP-ribose completely abolished OKT3 induced Ca^{2+} signalling in T-lymphocytes as previously shown by Guse *et al.* (1999). Furthermore, [^{3}H]-ryanodine binding stimulated by cADP-

Table 1 Summary of inhibition experiments for cADP-ribose, Ins(1,4,5)P_3 and suramin-induced Ca^{2+} release in Jurkat T-lymphocytes

Agonist	*Control*	*Ruthenium red*	*Ins(1,4,6) PS_3*	*1. 8-Br-cADP-ribose* *2. 8-NH_2-cADP-ribose* *3. 8-OCH_3-cADP-ribose*
cADP-ribose	$+^{a,b}$	$-^{a}$	$+^{b}$	1. $-^{a}$ 2. $-^{a,b}$ 3. $-^{c}$
Ins(1,4,5)P_3	$+^{a,b}$	$+^{a}$	$-^{b}$	1. $+^{a}$ 2. $+^{a}$ 3. $+^{c}$
Suramin	+*	−*	+*	1. n.d. 2. n.d. 3. +*
$Suramin_{extra}$	−*	n.d.	n.d.	n.d.

Fura-2 loaded T-lymphocytes were exposed to the indicated agonists in the absence and presence of inhibitors, like ruthenium red, Ins(1,4,6)PS_3 8-Br-cADP-ribose, 8-NH_2-cADP-ribose of 8-OCH_3-cADP-ribose. All agonists were applied to the intracellular side, however, $suramin_{extra}$ corresponds to extracellular application of suramin. The symbol '+' indicates the elevation of the intracellular Ca^{2+} concentration, '−' indicates no elevation of intracellular Ca^{2+} concentrations. The abbreviation 'n.d.' refers to 'not done'. The small letters indicate the following references: [a](Guse *et al.*, 1995); [b](Guse *et al.*, 1997; [c](Guse *et al.*, 1999); *results given in this report.

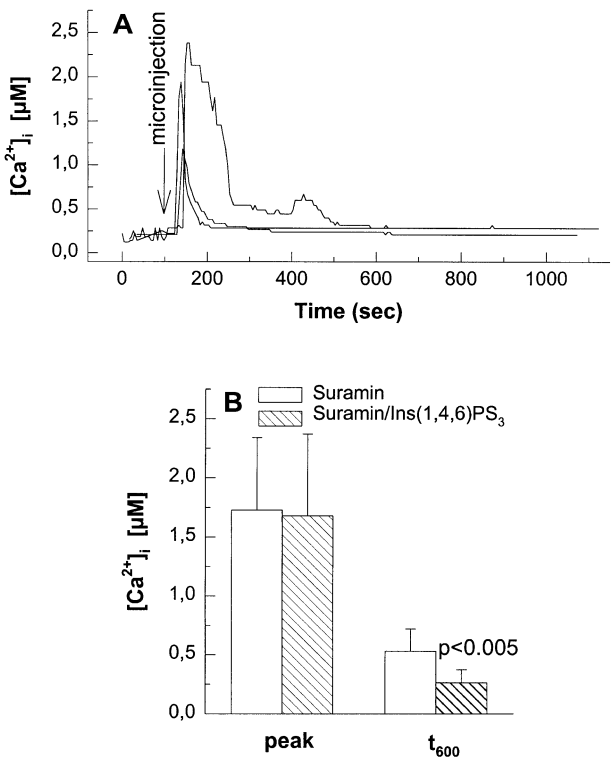

Figure 2 Co-injection of suramin and the IP_3-receptor antagonist, Ins(1,4,6)PS_3 accelerates the recovery to the resting $[Ca^{2+}]_i$. (A) 10 mM Suramin and 80 μM Ins(1,4,6)PS_3 were co-injected into Fura-2 loaded Jurkat T-lymphocytes. $[Ca^{2+}]_i$ was measured as described in the legend to Figure 1. Three representative $[Ca^{2+}]_i$ traces are given. (B) The $[Ca^{2+}]_i$ of the peak and t_{600} value were summarized from microinjection experiments with 10 mM suramin or co-injection of 10 mM suramin and 80 μM Ins(1,4,6)PS_3. The bars represent the mean and standard deviation of nine independent experiments.

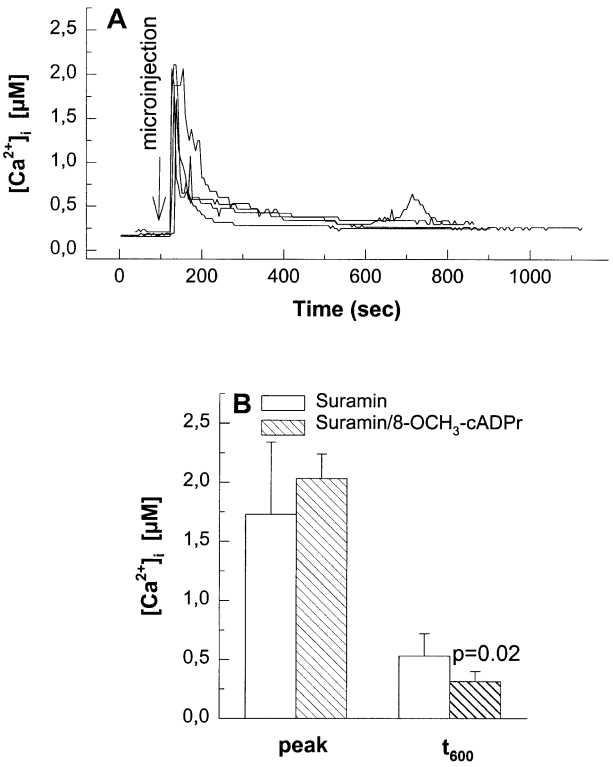

Figure 3 The cyclic ADP-ribose antagonist, 8-OCH_3-cADP-ribose, is not able to overcome the suramin induced elevation of $[Ca^{2+}]_i$. (A) Co-injection of 10 mM suramin and 100 μM 8-OCH_3-cADP-ribose into Fura-2 loaded Jurkat T-lymphocytes was carried out as indicated in the legend to Figure 1 to obtain the given traces for $[Ca^{2+}]_i$. (B) Analogous to Figure 2B the peak and t_{600} values of the $[Ca^{2+}]_i$ were summarized from microinjection experiments with 10 mM suramin ($n=15$) or co-injection of 10 mM suramin and 100 μM 8-OCH_3-cADP-ribose ($n=6$). The bars represent the mean and the corresponding standard deviation.

ribose in a $10{,}000 \times g$ membrane fraction from Jurkat T-lymphocytes was suppressed in the presence of 20 μM of 8-OCH_3-cADP-ribose (Guse *et al*., 1999). Co-injection of 10 mM suramin with 100 μM 8-OCH_3-cADP-ribose had no effect on the mean peak $[Ca^{2+}]_i$ (Figure 3B). However, the t_{600} values significantly were reduced from 0.53 ± 0.19 μM to 0.31 ± 0.08 μM [Ca2+]$_i$ ($P=0.023$) (Figure 3B). These experiments imply that suramin exerts the RyR opening independent from the cADP-ribose binding site.

Discussion

The primary event in mammalian immune response to antigen exposure is the clonal activation of antigen-specific T-lymphocytes. The capability of T-lymphocytes to recognize and respond to antigenic stimuli is possible through the T-cell antigen receptor. Upon T-cell receptor activation multiple signalling cascades are activated (Berridge, 1997). Ca^{2+} mobilization is initiated by the generation of the second messengers IP_3 and cADP-ribose (Guse, 1999; Guse *et al*., 1999) and leads to the nuclear factor of activated T-cells (NF-AT)-dependent expression of interleukin-2 and chemokines (Alberola *et al*., 1997; Ward & Westwick, 1998).

We have previously shown that the RyR is an essential player in T-lymphocyte activation. According to RT–PCR the RyR of T-cells can be classified as a type 3 RyR (Guse *et al*., 1999). In the present study we exploited the fact that the RyR type3 was the sole isoform present in Jurkat T-lymphocytes (Hakamata *et al*., 1994; Guse *et al*., 1999). Therefore these cells represent an ideal tool to investigate the pharmacological regulation of this Ca^{2+} channel and the role of this Ca^{2+} store.

The trypanocidal drug suramin is an agonist on the type 1 and type 2 RyR present in skeletal and cardiac muscle, respectively (Hohenegger *et al*., 1996). For the RyR type 1 we recently showed that suramin antagonized calmodulin effects in a competitive manner (Klinger *et al*., 1999). Furthermore the purified type1 RyR immobilized on a calmodulin-sepharose matrix was eluted by suramin. Taken together, these results argue for an agonistic effect of suramin mediated *via* a calmodulin binding site. Calmodulin binding sites are conserved in the type 3 RyR (Leeb & Brenig, 1998). We have therefore applied suramin in increasing concentrations using the microinjection method in order to circumvent its poor membrane permeability. Our observations show that suramin induced a specific activation of the RyR based on the following arguments (for overview see Table 1): (i) extracellular application of suramin did not alter $[Ca^{2+}]_i$; (ii) co-injection of 10 mM suramin with 2 μM ruthenium red completely abolished the suramin induced Ca^{2+} transient; (iii) the suramin induced Ca^{2+} transients were strictly dose-dependent (Figure 1B,C). Small molecular mass proteins in the injection pipette are expected to be diluted in the cytosol at least 50 fold (Guse *et al*., 1997).

Translation of this dilution factor results in an EC_{50} for suramin of 70–120 μM in the cell. This value is well within the

range which was required for the RyR type 1 to promote [^{3}H]-ryanodine binding or single channel recording of the purified receptor (Hohenegger *et al*., 1996). The dose-response curve for suramin is very steep, resulting in a Hill coefficient close to four. The ryanodine receptor exists as a homotetramer which shows cooperativity for most of the RyR ligands (Zucchi & Ronca-Testoni, 1997). This assumption implies that this channel opens according to a threshold activation. Previous observations in single channel recordings of the purified RyR type1 showed periods of continuous activation upon suramin addition for up to 2 min (Hohenegger *et al*., 1996). A similar behaviour of the T-cell RyR may partially explain the fact that the suramin induced Ca^{2+} signals in Jurkat T-lymphocytes are accompanied by sustained elevated $[Ca^{2+}]_i$ for more than 10 min (Figure 1A).

Emmick *et al*. (1994) showed that suramin inhibited the Ca^{2+}-ATPase of the rabbit skeletal muscle sarcoplasmic reticulum. Thus, sustained suramin-induced Ca^{2+} signals may also be due to an inhibitory effect of suramin on the Ca^{2+}-ATPase of the endoplasmic reticulum. However, we exclude this interpretation because the t_{600} values were significantly reduced in the presence of the antagonists for $InsP_3$ or cADP-ribose, which is unlikely due to reversal of the inhibition of the Ca^{2+}-ATPase of the endoplasmic reticulum in Jurkat T-lymphocytes (Figures 2B and 3B).

However, how can the inhibition of the sustained phase of suramin-stimulated Ca^{2+} signalling by Ins(1,4,6)PS_3 and 8-OCH_3-cADP-ribose be explained? Presumably, there was an opening of the RyR directly after the microinjection of suramin, due to the high local suramin concentrations. The subsequent dilution of suramin within the cell then slowly terminated this primary effect. In addition, as a result of the high $[Ca^{2+}]_i$, there was further Ca^{2+}-release by both, $InsP_3$-receptors and RyR's activated by basal $InsP_3$ and cADP-ribose plus high $[Ca^{2+}]_i$. Both, the RyR type 3 and the $InsP_3$-receptors type 1 show a bell-shaped Ca^{2+} activation curve and the appropriate Ca^{2+} levels sensitize the receptors to channel openers (Murayama & Ogawa, 1996; Hagar *et al*., 1998). Both receptors were activated at submicromolar Ca^{2+} concentrations. These properties may propagate the signal for a slow return of $[Ca^{2+}]_i$ to basal levels (Figure 1A). Since it is not possible to detect the basal $InsP_3$ and cADP-ribose levels on single cell levels in the absence or presence of microinjected suramin this interpretation is obviously speculative. However, by the addition of the antagonist (i.e. either Ins(1,4,6)PS_3 or 8-OCH_3-cADP-ribose) the sustained $[Ca^{2+}]_i$ elevation was inhibited. Therefore, these antagonists lead to a considerably shortened Ca^{2+} signal (Figures 2A and 3A).

Similar to suramin, caffeine also activates the type1 and 2 RyR (Meissner, 1994; Zucchi & Ronca-Testoni, 1997, Klinger *et al*., 1999). However, the response of type3 RyR to caffeine is still controversial. Giannini *et al*. (1992) and Hakamata *et al*. (1994) reported that caffeine did not evoke an increase in $[Ca^{2+}]_i$ in mink lung epithelial cells and Jurkat T-lymphocytes, respectively, although a type 3 RyR was present in these cells. In contrast to these observations, Murayama & Ogawa, (1996) could clearly find a Ca^{2+}-dependent increase in [^{3}H]-ryanodine binding upon addition of caffeine in rabbit brain membranes enriched in RyR type 3. These discrepancies in response to caffeine and the fact that microinjection of caffeine will lead to diffusion to the extracellular solution makes this compound not comparable to suramin. In summary, evidence is provided for an agonistic effect of suramin on T-cell RyR opening and the possibility of immunopharmacological modulation of the T-cell activity *via* the cADP-ribose Ca^{2+}-signalling pathway.

This work was supported by grants of the Austrian National Bank (No. 6644) and the Büro zur Förderung von Auslandsbeziehungen (to M. Hohenegger), the Deutsche Forschungsgemeinschaft (No.: Gu 360/2-2 and 2-3 to A.H. Guse and G.W. Mayr) and the Wellcome Trust (research collaboration grant No.: 051326 to A.H. Guse and B.V.L. Potter; and a programme grant No.: 045491 to B.V.L. Potter).

References

ALBEROLA, I.J., TAKAKI, S., KERNER, J.D. & PERLMUTTER, R.M. (1997). Differential signalling by lymphocyte antigen receptors. *Annu. Rev. Immunol.,* **15,** 125 – 154.

ASHAMU, G.A., SETHI, J.K., GALIONE, A. & POTTER, B.V.L. (1997). Roles for adenosine ribose hydroxyl groups in cyclic adenosine 5′-diphosphate ribose-mediated Ca^{2+} release. *Biochemistry,* **36,** 9509 – 9517.

BERRIDGE, M.J. (1997). Lymphocyte activation in health and disease. *Crit. Rev. Immunol.,* **17,** 155 – 178.

CLAPHAM, D.E. (1995). Calcium Signalling. Cell, **80,** 259 – 268.

CRABTREE, G.R. & CLIPSTONE, N.A. (1994). Signaltransmission between the plasma membrane and the nucleus of T lymphocytes. *Ann. Rev. Biochem.,* **63,** 1045 – 1083.

EMMICK, J.T., KWON, S., BIDASEE, K.R., MESCH, K.T. & BESCH, H.R. (1994). Dual effect of suramin on calcium fluxes across sarcoplasmic reticulum vesicle membranes. *J. Pharmacol. Exp. Ther.,* **269,** 717 – 724.

FRUEN, B.R, MICKELSON, J.R., SHOMER, N.H., VELEZ, P. & LOUIS, C.F. (1994). Cyclic ADP-ribose does not affect cardiac or skeletal muscle ryanodine receptors. *FEBS Lett.,* **352,** 123 – 126.

GIANNINI, G., CLEMENTI, E., CECI, R., MARZIALI, G., & SORRENTINO, V. (1992). Expression of a ryanodine receptor-Ca^{2+} Channel that is regulated by TGF-β. *Science,* **257,** 91 – 94.

GIANNINI, G., CONTI, A., MAMMARELLA, S., SCROBOGNA, M. & SORRENTINO, V. (1995). The ryanodine receptor/calcium channel genes are widely and differentially expressed in murine brain and peripheral tissues. *J. Cell Biol.,* **128,** 893 – 904.

GUSE, A.H. (1998). Ca^{2+} signalling in the T-lymphocytes. *Crit. Rev. Immunol.,* **18,** 419 – 448.

GUSE, A.H. (1999). Cyclic ADP-ribose: a novel Ca^{2+} mobilising second messenger. *Cell Signal.,* **11,** 309 – 316.

GUSE, A.H., BERG, I., DASILVA, C.P., POTTER, B.V.L. & MAYR, G.W. (1997). Ca^{2+} entry induced via cyclic ADP-ribose in intact T-lymphocytes. *J. Biol. Chem.,* **272,** 8546 – 8550.

GUSE, A.H., DASILVA, C.P., BERG, I., SKAPENKO, A.L., WEBER, K., HEYER, P., HOHENEGGER, M., ASHAMU, G.A., SCHULZE-KOOPS, H., POTTER, B.V.L. & MAYR, G.W. (1999). Regulation of Ca^{2+}-signalling in T-lymphocytes by the second messenger cyclic ADP-ribose. *Nature,* **398,** 70 – 73.

GUSE, A.H., DASILVA, C.P., EMMRICH, F., ASHAMU, G.A., POTTER, B.V.L. & MAYR, G.W. (1995). Characterization of cyclic adenosine diphosphate-ribose-induced Ca^{2+} -release in T lymphocyte cell lines. *J. Immunol.,* **155,** 3353 – 3359.

GUSE, A.H., ROTH, E. & EMMRICH, F. (1993). Intracellular Ca^{2+} pools in Jurkat T-lymphocytes. *Biochem. J.* **291,** 447 – 451.

HAGAR, R.E., BURGSTAHLER, A.D., NATHANSON, M.H., & EHRLICH, B.E. (1998). TypeIII InsP3 receptor channel stays open in the presence of increased calcium. *Nature,* **396,** 81 – 84.

HAKAMATA, Y., NAKAI, J., TAKESHIMA, H., & IMOTO, K. (1992). Primary structure and distribution of a novel ryanodine receptor/calcium release channel from rabbit brain. *FEBS Lett.,* **312,** 229 – 235.

HAKAMATA, Y., NISHIMURA, S., NAKAI, Y. J., NAKSHIMA, Y., KITA, T. & IMOTO, K. (1994). Involvement of the brain type ryanodine receptor in T-cell proliferation. *FEBS Lett.,* **352,** 206 – 210.

HOHENEGGER, M., MATHYASH, M., POUSSU, K., HERRMANN-FRANK, A., SARKÖZI, S., LEHMANN-HORN, F. & FREISSMUTH, M. (1996). Activation of the skeletal muscle ryanodine receptor by suramin and suramin analogs. *Mol. Pharmacol.,* **50,** 1443–1453.

JAYARAMAN, T., ONDRIASOVE, E., ONDRIAS, K., HARNICK, D.J. & MARKS, A.R. (1995). The inositol 1,4,5-triphosphate receptor is essential for T-cell receptor signalling. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 6007–6011.

KLINGER, M., FREISSMUTH, M., NICKEL, P., STÄBLER-SCHWARZ-BART, M., KASSACK, M., SUKO, J. & HOHENEGGER, M. (1999). Suramin and suramin analogs activate skeletal muscle ryanodine receptor via a calmodulin binding site. *Mol. Pharmacol.,* **55,** 462–472.

LEEB, T. & BRENIG, B. (1998). cDNA cloning and sequencing of the human ryanodine receptor type 3 (RyR3) reveals a novel alternative splice site in the RyR3 gene. *FEBS Lett.,* **423,** 367–370.

MARKS, A.R. (1997). Intracellular calcium-release channels: regulators of cell life and death. *Am. J. Physiol.,* **272,** H597–H605.

MEISSNER, G. (1994). Ryanodine receptor/Ca^{2+} release channels and their regulation by endogenous effectors. *Annu. Rev. Physiol.,* **56,** 485–508.

MILLS, S.J. & POTTER, B.V.L. (1996). Synthesis of D- and L-myo-inositol 1,4,6-trisphosphate, regioisomers of a ubiquitous second messenger. *J. Org. Chem.,* **61,** 8980–8987.

MILLS, S.J., RILEY, A.M., MURPHY, C.T., BULLOCK, A., WESTWICK, J., & POTTER, B.V.L. (1995). Myo-inositol 1,4,6-trisphosphorothioate and myo-inositol 1,3,4-trisphosphorothioate: New synthetic Ca^{2+}-mobilising partial agonists at the inositol 1,4,5-trisphosphate receptor. *Bioorg. Med. Chem. Lett.,* **5,** 203–208.

MURAYAMA, Y. & OGAWA, Y. (1996). Properties of ryr3 ryanodine receptor isoform in mammalien brain. *J. Biol. Chem.,* **271,** 5079–5084.

NAKASHIMA, Y., NISHIMURA, S., MAEDA, A., BARSOUMIAN, E.L., HAKAMATA, Y., NAKAI, J., ALLEN, P.D., IMOTO, K. & KITA, T. (1997). Molecular cloning and characterisation of a human brain ryanodine receptor. *FEBS Lett.,* **417,** 157–162.

SUGIYAMA, T., FURUYA, A., MONKAWA, T., YAMAMOTO-HINO, M., SATOH, S., OHMORI, K., MIYAWAKI, A., HANAI, N., MIKOSHIBA, K. & HASEGAWA, M. (1994). Monoclonal antibodies distinctively recognizing the subtypes of inositol 1,4,5-trisphosphate receptor: application to the studies on inflammatory cells. *FEBS-Lett.,* **354,** 149–154.

WARD, S.G. & WESTWICK, J. (1998). Chemokines: understanding their role in T-lymphocyte biology. *Biochem. J.,* **333,** 457–470.

ZUCCHI, R. & RONCA-TESTONI, S. (1997). The sarcoplasmic reticulum Ca^{2+}channel/ ryanodine receptor: Modulation by endogenous effectors, drugs and disease states. *Pharmacol. Rev.,* **49,** 1–48.

(Received September 6, 1999
Accepted September 10, 1999)