tissues, heart, liver and kidney, the PBR is also known to be highly expressed in blood cells. In this work, we demonstrate by flow cytometry experiments, that SSR180575, at low concentrations, is able to protect polymorphonuclear leukocytes (PMNs) against TNFα-induced apoptosis in whole blood. Thus, in a new context, SSR180575 again shows potent anti-apoptotic properties. Moreover, TNFα-induced PMN apoptosis appears to be a good surrogate marker for determining SSR180575 blood availability and activity in treated patients.

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15P.2 The dynamics of mitochondrial Ca^{2+} fluxes monitored with targeted aequorin

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Mitochondrial Ca^{2+} fluxes play a very important role in cell physiology. In spite of the large negative potential of the mitochondrial matrix, mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_M$) is low under resting conditions because of the operation of systems able to extrude Ca^{2+} from mitochondria in exchange by Na^+ or H^+. During cell activation, the increase in cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) triggers the opening of the mitochondrial Ca^{2+} uniporter and large amounts of Ca^{2+} flow through this channel into the mitochondrial matrix. We have used here low-Ca^{2+}-affinity aequorin to investigate in permeabilized cells the behaviour of mitochondrial Ca^{2+} fluxes under different conditions of $[Ca^{2+}]_c$, $[Na^+]$ and temperature to obtain a clearer picture of mitochondrial $[Ca^{2+}]$ homeostasis. The rate of Ca^{2+} release from mitochondria increased linearly with mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_M$). Na^+-dependent Ca^{2+} release was predominant al low $[Ca^{2+}]_M$ but saturated at $[Ca^{2+}]_M$ around 400mM, while Na^+-independent Ca^{2+} release was very slow at $[Ca^{2+}]_M$ below 200 mM, and then increased at higher $[Ca^{2+}]_M$, perhaps through the opening of a new pathway. Half-maximal activation of Na^+-dependent Ca^{2+} release occurred at 5-10 mM $[Na^+]$, within the physiological range of cytosolic $[Na^+]$. Ca^{2+} entry rates were comparable in size to Ca^{2+} exit rates at cytosolic $[Ca^{2+}]_c$ below 7 mM, but the rate of uptake was dramatically accelerated at higher $[Ca^{2+}]_c$. As a consequence, the presence of $[Na^+]$ considerably reduced the rate of $[Ca^{2+}]_M$ increase at $[Ca^{2+}]_c$ below 7 mM, but its effect was hardly appreciable at 10 mM $[Ca^{2+}]_c$. Exit rates were more dependent on the temperature than uptake rates, thus making the $[Ca^{2+}]_M$ transients to be much more prolonged at lower temperature. Our kinetic data suggest that mitochondria have little high affinity Ca^{2+} buffering. Comparison of our results with previous data on total mitochondrial Ca^{2+} fluxes indicate that the mitochondrial Ca^{2+} bound/Ca^{2+} free ratio is around 100 for most of the observed $[Ca^{2+}]_M$ range and suggest that massive phosphate precipitation can only occur when $[Ca^{2+}]_M$ reaches the millimolar range. In conclusion, our data reveal mitochondria as a highly dynamic compartment in terms of Ca^{2+} homeostasis, able to take up and release Ca^{2+} fast enough to follow the cytosolic Ca^{2+} transients, and also able to undergo reversible variations in $[Ca^{2+}]_M$ that could span up to four orders of magnitude, from 100 nM to 1 mM.

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15P.3 Is nitric oxide synthase present in mitochondria?

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In addition to the three known forms of nitric oxide synthase (NOS) in the heart, it has been proposed that NOS is also present in mitochondria. However, studies are controversial due to the possibility of contamination by non-mitochondrial NOS, and because none of the known forms of NOS contain a mitochondrial targeting sequence. We investigated whether NOS was present in isolated mitochondria using antibodies against all 3 forms of NOS (endothelial NOS, inducible NOS and neuronal NOS). Crude fractions of heart and liver mitochondria were obtained by differential centrifugation, and 35% Percoll used to obtain highly-purified mitochondria, as tested using antibodies against subcellular marker proteins: cyclophilin D — mitochondrial marker; monocarboxylate transporter-1 — plasma membrane marker; ryanodine receptor — sarcoplasmic reticulum marker (heart mitochondria only); and catalase — peroxisomal marker (liver only). Western blotting using antibodies against eNOS and iNOS revealed that these isoforms were not present in either heart or liver purified mitochondria (whereas whole heart or liver lysate tested positive). We used 5 different antibodies against nNOS, and again failed to detect anything in purified heart mitochondria. In purified liver mitochondria one of the nNOS antibodies revealed the presence of a band at the correct molecular weight. However, subsequent analysis by mass spectrometry revealed that this was the enzyme carbamoyl phosphate synthase, and not an isoform of NOS. Although our results show that mitochondria do not contain a specific form of NOS, it remains possible that NO from one of the known forms of NOS can regulate mitochondrial function.

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15P.4 Effect of long-term exercise training on the sensitivity of calcium-induced mitochondrial permeability transition pore opening and uncoupling protein 3 expression

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Exercise training improves cardiovascular capacity and reduces the risk of cardiovascular heart diseases. Exercise has the potential to reduce apoptosis through upregulation of protective stress-sensitive proteins including nuclear factor kappa-B, insulin-like growth factor, and heat shock proteins [1]. Mitochondrial permeability transition pore (MPTP) opening plays a significant role in the transition of mitochondria from a physiological condition to induction of cell death [2]. In the heart, MPTP opening was shown to occur during reperfusion after ischemia and to be involved in contractile dysfunction and tissue injury [3]. However, the mechanisms by which