# The inhibitory effect of ionophore A23187 on the hydrolysis of fluorescein diacetate by human lymphocytes

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Fluorescence measured after the intracellular hydrolysis of fluorescein diacetate (FDA) may be used as an indicator of cell viability and membrane integrity (Rotman & Papermaster, 1966). Moreover, in lymphocytes from normal subjects the fluorescence polarization of intracellular fluorescein is decreased after phytohaemagglutinin (PHA) treatment, whereas in lymphocytes from cancer patients no such effect of PHA is observed ('SCM test') (Cercek, Cercek & Franklin, 1974; Cercek & Cercek, 1977; Kreutzmann, Fliedner, Galla & Sackmann, 1978).

In view of the possible importance of kinetic phenomena (Epstein, Norman, Pinkel & Udkoff, 1977) in the SCM test we previously studied the effects of PHA on the total fluorescence produced by lymphocyte hydrolysis of FDA and found that the rate of increase in fluorescein fluorescence is markedly decreased by PHA pretreatment (Mullen & Campbell, 1979). To further investigate this inhibitory action of PHA we have now studied the effect of A23187 on FDA hydrolysis considering that both PHA induced lymphocyte mitogenesis (Maino, Green & Crumpton, 1974) and the SCM test (Cercek & Cercek, 1977) are calcium dependent.

Washed (×2) lymphocytes from healthy volunteer subjects (mean age 28 years) were suspended in Eagle's basal medium ($4 \times 10^6$ cells/ml). One ml of this suspension was incubated for 30 min at 37°C after the addition of 0.1 ml of A23187 (8.25 μg/ml) in medium containing 4% ethanol vehicle. Control cells received 0.1 ml of medium containing 4% alcohol. At zero time 200 μl of the incubated cell suspension were added to 3.8 ml of FDA (2.5–10 μM) solution in phosphate buffered saline. Fluorescence intensity (F.I.) readings (excitation, 473 nm; emission, 510 nm) were obtained at 15 s intervals over an 8.5 min period. The ionophore did not affect cell viability as measured by trypan blue exclusion.

Initial experiments ($n = 8$) using a single FDA concentration (2.5 μM) indicated that the total increase in fluorescence intensity with time was much reduced in A23187 treated lymphocytes compared with controls (mean values were 2.8 and 4.0 F.I. units/min/$1.8 \times 10^5$ cells/ml, respectively; paired $t$-test. $P = 0.004$). Although the overall kinetics of FDA hydrolysis by living cells are complex (Sontag, 1977), the increase in total fluorescence is describable in terms of Michaelis-Menten kinetics over the range of FDA concentrations employed. Like PHA (Mullen & Campbell, 1979), A23187 appears to noncompetitively inhibit fluorescein production. In ten experiments the mean Michaelis-Menten parameters ± s.e. mean for total fluorescence production were: $K_m = 6.3 \pm 1.1$; $V_{max} = 12.5 \pm 1.9$ in controls compared to $K_m = 6.9 \pm 1.3$; $V_{max} = 4.9 \pm 0.7$ for A23187 treated cells {in μmols/l and F.I. units/min/$1.8 \times 10^5$ cells/ml, respectively—$V_{max}$ being significantly ($P < 0.001$, paired $t$-test) lower in A23187 treated cells}.

In addition to its mitogenic capacity (Maino, Green & Crumpton, 1974), A23187 also appears to mimic the inhibitory action of PHA on the fluorochromatic (Rotman & Papermaster, 1966) process.

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