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Δ^9-Tetrahydrocannabinol activates $[Ca^{2+}]_i$ increases partly sensitive to capacitative store refilling

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Abstract

Δ^9-Tetrahydrocannabinol induces $[Ca^{2+}]_i$ increases in DDT_1MF-2 smooth muscle cells. Both Ca^{2+} entry and release from intracellular Ca^{2+} stores were concentration dependently activated. The Ca^{2+} entry component contributed most to the increases in $[Ca^{2+}]_i$. Stimulation with Δ^9-tetrahydrocannabinol after functional downregulation of intracellular Ca^{2+} stores by longterm thapsigargin treatment, still induced a major Ca^{2+} entry and a minor Ca^{2+} release component. Thapsigargin sensitive influx and release were selectively inhibited by the cannabinoid CB_1 receptor antagonist SR141716A. No effects on $[Ca^{2+}]_i$ were obtained after stimulation with the CB_2 receptor agonist palmitoylethanolamide. This study is the first demonstration of (1) Ca^{2+} release from thapsigargin sensitive intracellular stores and capacitative Ca^{2+} entry via CB_1 receptor stimulation and of (2) an additional Δ^9-tetrahydrocannabinol induced thapsigargin insensitive component, mainly representing Ca^{2+} influx which is neither mediated by CB_1 nor CB_2 receptor stimulation.

Keywords: Δ^9-Tetrahydrocannabinol; $[Ca^{2+}]_i$; DDT_1MF-2 cell

Cannabinoid receptors are member of the G-protein coupled receptor family and stimulation was shown to lead to inhibition of adenylate cyclase, activation of inwardly rectifying K^+ channels and inhibition of voltage dependent Ca^{2+} channels (Felder et al., 1995). In response to neurotransmitters or growth factors many receptors of this family also transduce essential signals for cellular functioning via changes in $[Ca^{2+}]_i$ by activating for instance the phospholipase C cascade. However, to date only a few ambiguous results were reported on $[Ca^{2+}]_i$ after cannabinoid receptor stimulation. Anandamide, an endogenous ligand for the CB_1 receptor subtype increases $[Ca^{2+}]_i$ at concentrations above 10 μM, probably through a non-receptor mediated mechanism (Felder et al., 1993), while rapid transient elevations of $[Ca^{2+}]_i$ were elicited by the CB_2 receptor agonist 2-arachidonylglycerol. However, these latter transients could be blocked by the specific CB_1 receptor antagonist SR141716A (Sagiura et al., 1996).

To investigate whether cannabinoid receptor stimulation induces receptor mediated changes in Ca^{2+} mobilization from intracellular stores and/or Ca^{2+} entry, we measured $[Ca^{2+}]_i$ using Fura-2 fluorometry in DDT_1MF-2 smooth muscle cells. These cells are appropriate, since they respond to many different receptor ligands with increases in $[Ca^{2+}]_i$ and do not express voltage-dependent Ca^{2+} channels (Molleman et al., 1991). Cells were loaded with 2 μM Fura-2 acetoxymethyl ester at 22°C, for 30 min in the dark. Fluorescence was measured at 37°C and $[Ca^{2+}]_i$ calculated as described previously (Sipma et al., 1995). A basal $[Ca^{2+}]_i$ of 148 ± 19 nM ($n = 48$) was found. In contrast to the CB_2 receptor agonist palmitoylethanolamide which failed to induce a change in $[Ca^{2+}]_i$ (148 ± 20 nM; $n = 4$), the non-selective cannabinoid receptor agonist Δ^9-tetrahydrocannabinol (Felder et al., 1995) produced a concentration-dependent increase in $[Ca^{2+}]_i$ (Fig. 1A, a). The Ca^{2+} response obtained in normal buffer solution consisted of a transient first phase followed by a sustained increase, comparable to responses observed after stimulation of other receptors in DDT_1MF-2 cells (Den Hertog et al., 1992; Sipma et al., 1995). To establish the relative contribution of transient intracellular Ca^{2+} release and sustained Ca^{2+} influx from extracellular space to the $[Ca^{2+}]_i$ change, the experiment was repeated in Ca^{2+} free buffer solution including 1.5 mM EGTA. Δ^9-Tetrahydrocannabinol released Ca^{2+} from internal stores concentra-

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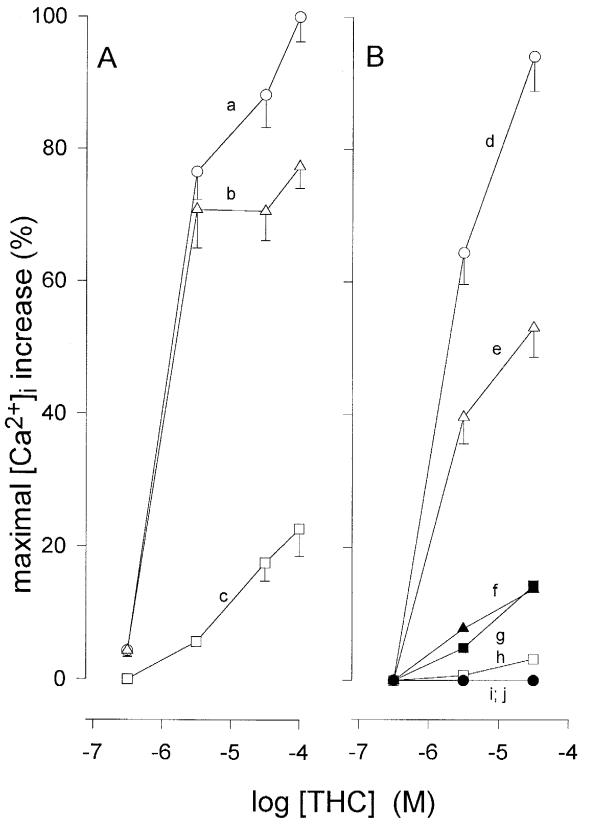

Fig. 1. Δ^9-Tetrahydrocannabinol effects on $[Ca^{2+}]_i$ in DDT_1MF-2 cells. Panel A: curve (a), changes in total $[Ca^{2+}]_i$ as obtained in normal external medium; curves (b) and (c), influx and release component, respectively. The release component was obtained by measuring maximum values in Ca^{2+} free medium with 1.5 mM EGTA included. The influx component obtained by substraction the releasable values from the total values. Panel B: Effects obtained after pretreatment of the cells with thapsigargin (1 μM) for 48 h; curve (d), changes in total $[Ca^{2+}]_i$ as obtained in normal external medium; curve (e), thapsigargin insensitive influx; curve (f) and (g), thapsigargin sensitive influx and release, respectively; curve (h), thapsigargin insensitive release; curves (i) and (j), superimposed curves showing complete inhibition of the thapsigargin sensitive influx and release (f and g) after pretreatment with SR141716A (1 μM, 1 min). All values were normalized to the maximum total $[Ca^{2+}]_i$ value as obtained with 100 μM Δ^9-tetrahydrocannabinol (378 ± 56 nM, $n = 6$). Each symbol represents the mean ± S.E.M. from at least 4 separate experiments.

tion dependently (Fig. 1A, c). The difference observed between the changes in $[Ca^{2+}]_i$ in the presence and absence of extracellular Ca^{2+} represent a substantial Ca^{2+} influx component (Fig. 1A, b). In accordance readdition of 2.5 mM Ca^{2+} after a Δ^9-tetrahydrocannabinol (100 μM) response under extracellular free Ca^{2+} conditions, completely restored the Ca^{2+} entry component ($98 \pm 5\%$; $n = 4$). Surprisingly, the Ca^{2+} release component consisted of two phases: a pronounced transient second phase developed (maximum values reached after 31 ± 3 s; $n = 6$) before the first transient phase was returned to basal values (maximum values reached after 26 ± 3 s; $n = 6$).

To further investigate the nature of these intracellular compartments, Ca^{2+} stores were functional downregulated by longterm treatment with the endoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin (1 μM, 48 h; Short et al., 1993). The first phase after Δ^9-tetrahydrocannabinol stimulation under extracellular Ca^{2+} free conditions was not affected, but the second phase was completely abolished. This indicates the presence of two intracellular Ca^{2+} stores susceptible to stimulation with Δ^9-tetrahydrocannabinol, one of which is thapsigargin insensitive (Fig. 1B, h) and one thapsigargin sensitive (Fig. 1B, g). Apparently Δ^9-tetrahydrocannabinol is able to release Ca^{2+} from two distinguishable agonist sensitive Ca^{2+} stores existent in DDT_1MF-2 cells (Den Hertog et al., 1992). Due to down-regulation of the Ca^{2+} stores evidently also the Ca^{2+} entry component is affected. The majority of Ca^{2+} entry is thapsigargin insensitive (Fig. 1B, e) and about 15% is thapsigargin sensitive (Fig. 1B, f), representing the so called store-dependent capacitative Ca^{2+} entry (Putney, 1986).

Pretreatment with the CB_1 receptor antagonist SR141716A (1 μM, 1 min) did neither affect thapsigargin insensitive Ca^{2+} influx nor Ca^{2+} release (data not shown), but selectively abolished the thapsigargin sensitive Ca^{2+} release and the capacitative Ca^{2+} entry (Fig. 1B, i and j). These results were confirmed by Ca^{2+} readdition experiments in the presence of SR141716A in which Δ^9-tetrahydrocannabinol (32 μM) responses were restored to $78 \pm 3\%$ ($n = 6$) compared to control values.

The principle findings of the present study performed in DDT_1MF-2 smooth muscle cells are the first demonstration of (1) Ca^{2+} release from thapsigargin sensitive intracellular stores and capacitative Ca^{2+} entry via CB_1 receptor stimulation and of (2) an additional Δ^9-tetrahydrocannabinol induced thapsigargin insensitive component, mainly representing Ca^{2+} influx which is neither mediated by CB_1 nor CB_2 receptor stimulation.

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