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SPECIAL REPORT

Potentiation by tumour necrosis factor-α of calcium signals induced by bradykinin and carbachol in human tracheal smooth muscle cells

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The effect of tumour necrosis factor-α (TNFα) on the increase in cytosolic free calcium ($[Ca^{2+}]_i$) induced by carbachol and bradykinin (BK) was investigated in human tracheal smooth muscle cells in culture (TSMC). BK (10^{-12}–10^{-9} M) and carbachol (10^{-7}–10^{-3} M) produced a concentration-dependent increase in $[Ca^{2+}]_i$ ($pD_2 = 10.73 \pm 0.05$ and 5.57 ± 0.03 respectively). The increase in $[Ca^{2+}]_i$ was significantly enhanced for both agonists in TNFα-treated cells (10 ng ml^{-1} for 24 h). However, pD_2 values were not modified (10.78 ± 0.03 and 5.62 ± 0.04 for BK and carbachol, respectively) suggesting that no change in the apparent receptor affinity occured. Thus, TNFα induced-alterations in Ca^{2+} homeostasis in human TSMC may be a key mechanism underlying airway hyperreactivity.

Keywords: Bradykinin; calcium; human tracheal smooth muscle cells; tumour necrosis factor-α; carbachol

Introduction *In vivo*, inhalation of tumour necrosis factor-α (TNFα) caused bronchial hyperresponsiveness in rats (Kips *et al.*, 1992). The precise mechanism underlying this enhancement of airway responsiveness remains to be elucidated, but an *in vitro* study indicates that TNFα can have a direct effect on tracheal smooth muscle (Pennings *et al.*, 1993). Such a mechanism may include alterations in the contractile properties of airway smooth muscle by TNFα and this is the basic hypothesis of the present study.

We have investigated whether TNFα modifies the subsequent response of human tracheal smooth muscle cells (TSMC) to bronchoconstrictors agonists. Since Ca^{2+} is a second messenger common to all airway smooth muscle cell activators, including bradykinin and carbachol (Murray & Kotlikoff, 1991; Marsh & Hill, 1993; Yang *et al.*, 1993), we studied the effect of pretreatment of human TSMC with TNFα on the $[Ca^{2+}]_i$ response to these agonists.

Methods Primary cultures of human TSMC were prepared as described previously (Kullman *et al.*, 1993). TSMC were cultured in DMEM/F12 medium supplemented with 10% foetal calf serum, 2 mM glutamine, 1% non-essential amino-acids, 5 μg ml^{-1} insulin, penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). All products were obtained from Gibco BRL (Cergy Pontoise, France).

Human TSMC at confluency were treated for 24 h with 10 ng ml^{-1} TNFα (Genzyme, Dako SA, Trappes) in the above described medium. Cells were washed with HEPES buffer containing (mM): NaCl 137.5, $CaCl_2$ 1.25, $MgCl_2$ 1.25, NaH_2PO_4 0.4, KCl 6, glucose 5.6, HEPES 10, 0.1% BSA (w/v); pH = 7.4 and incubated with 3 μM fura-2/AM in HEPES buffer (Sigma, St Louis, MO, U.S.A.) at 37°C for 45 min. The cells were trypsinized, washed and resuspended at 5×10^5 cells ml^{-1}. $[Ca^{2+}]_i$ was calculated with a Hitachi F2000 spectrofluorometer from the fluorescence intensities measured at 510 nm after excitation at both 340 and 380 nm. Statistical analysis was calculated by Student's *t* test.

Results Figure 1 shows the effect of BK on the increase in $[Ca^{2+}]_i$ in human TSMC treated or not with 10 ng ml^{-1} of TNFα. In control cells, 10^{-10} M BK induced a typical biphasic increase in $[Ca^{2+}]_i$ consisting of a fast transient phase followed by a sustained phase, i.e. $[Ca^{2+}]_i$ did not return to the basal level (Figure 1a). In TNFα pretreated

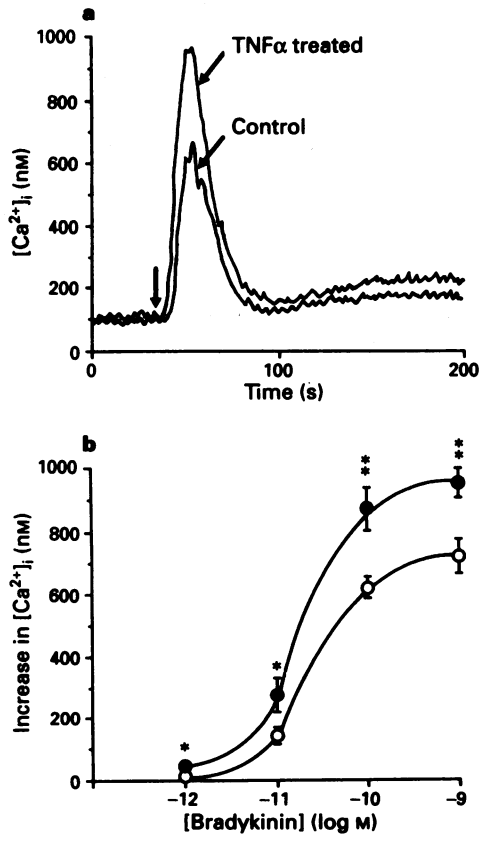

Figure 1 Effects of tumour necrosis factor-α (TNFα) pretreatment of human TSMC on the increase in $[Ca^{2+}]_i$ induced by BK. (a) Typical traces obtained with 10^{-10} M BK in untreated cells (Control) and TNFα-treated cells (10 ng ml^{-1} for 24 h). The arrow indicates the addition of BK (b) concentration-response curves to BK in untreated (○) and in TNFα-treated cells (●). Results are expressed as the net increase in $[Ca^{2+}]_i$ at the peak and values are means ± s.e.mean of four separate experiments. Significantly different from control: $P<0.05$*; $P<0.01$**.

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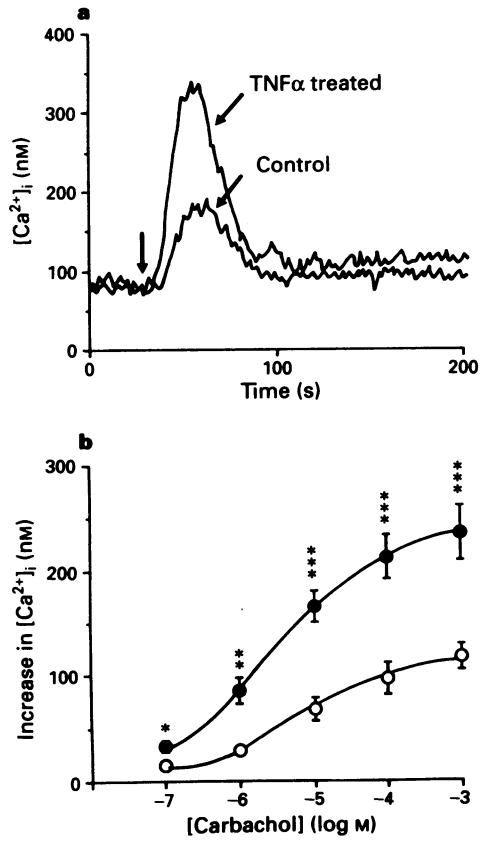

Figure 2 Effects of tumour necrosis factor-α (TNFα) pretreatment of human TSMC on the increase in $[Ca^{2+}]_i$ induced by carbachol. (a) Typical traces obtained with 10^{-3} M carbachol in untreated cells (Control) and TNFα-treated cells (10 ng ml^{-1} for 24 h). The arrow indicates the addition of carbachol. (b) Concentration-response curves to carbachol in untreated (○) and in TNFα-treated cells (●). Results are expressed as the net increase in $[Ca^{2+}]_i$ at the peak and values are means ± s.e.mean of four separate experiments. Significantly different from control: $P<0.05$*; $P<0.01$**; $P<0.001$***.

TSMC, both phases were increased (Figure 1a). BK induced a concentration-dependent increase in $[Ca^{2+}]_i$ between 10^{-12} and 10^{-9} M in the transient phase (Figure 1b) which was significantly potentiated upon TNFα treatment (Figure 1b). The maximum response in treated and untreated cells was reached at 10^{-9} M. pD_2 values were 10.73 ± 0.05 in control and 10.78 ± 0.03 in treated cells, showing that TNFα did not modify the sensitivity of the concentration-response curves. In addition, the sustained phase was also potentiated by 81, 60 and 38% at 10^{-11}, 10^{-10} and 10^{-9} M BK, respectively (results not illustrated).

Figure 2 shows the effect of carbachol on the increase in $[Ca^{2+}]_i$ in human TSMC treated or not with TNFα. Carbachol at 10^{-3} M induced a biphasic increase in $[Ca^{2+}]_i$ which was potentiated (for both phases) in TNFα-treated cells (Figure 2a). As shown in Figure 2b, TNFα significantly potentiated the transient phase for all concentrations of carbachol that were tested. The maximum increase was obtained at 10^{-3} M carbachol. pD_2 values were 5.57 ± 0.03 in control and 5.62 ± 0.04 in treated cells indicating that the sensitivity of the concentration-response curves are not modified. The sustained phase was also potentiated in TNFα-treated cells by 144, 106 and 86% for 10^{-6}, 10^{-4} and 10^{-3} M carbachol, respectively (results not illustrated).

Discussion To our knowledge, this study is the first demonstration of a direct effect of TNFα on human TSMC in culture by enhancing the $[Ca^{2+}]_i$ response to BK and carbachol. The pD_2 values remained unmodified suggesting that the cell sensitivity to these agonists is not affected by TNFα. Interestingly, a parallel can be drawn with the work of Pennings *et al.* (1993) who showed that pretreatment of guinea-pig trachea with TNFα increases the maximum contractile response to methacholine without changing the pD_2 values.

Such a potentiation could involve an over-expression of cell surface receptors, but this would imply an increase in the number of both BK and muscarinic receptors. Surprisingly, we found that a 24 h TNFα pretreatment of human TSMC decreased the specific binding sites number of [^{3}H]-quinuclidinylbenzilate (a muscarinic antagonist) by 37.8 ± 1.3% ($n = 3$) (Amrani, unpublished observations) ruling out the hypothesis of an increase in the number of receptors. Alternatively, TNFα might modify receptor-linked transduction mechanisms, such as G-protein and subsequent effector systems such as phospholipase C, both involved in the increase in $[Ca^{2+}]_i$ in response to carbachol and BK (Pyne & Pyne, 1993; Yang *et al.*, 1993). TNFα might also affect the expression of proteins involved in the regulation of intracellular Ca^{2+} stores and/or Ca^{2+}-influx.

In conclusion, our results demonstrate that human airway smooth muscle cells are a new target for TNFα. Alterations in Ca^{2+} handling by TNFα may represent a key mechanism underlying airway hyperreactivity.

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