Differential responses to histamine and cyclo-pentyladenosine in subclones of the DDTMF-2 cell line.

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The hamster vas deferens derived smooth muscle cell line, DDTMF-2, has been shown to possess functional histamine H1-receptors, stimulation of which leads to the production of the second messenger inositol 1,4,5-trisphosphate (IP3) and subsequent release of calcium from intracellular stores [1,2]. This cell line also possesses both adenosine A1- and A2-receptors which are coupled respectively negatively and positively to adenylate cyclase [3,4]. We have shown previously that stimulation of the A2-receptor can also lead to inositol phospholipid hydrolysis in this cell line [5].

In this study we have isolated and maintained a total of eight clonal lines (TW1-8) from a population of DDTMF-2 cells which we believe to be heterogeneous. The clonal cell lines were established using a combination of microscopy and single cell isolation techniques. To this end, a suspension of the DDTMF-2 cell line was diluted to give a theoretical cell density of 1 cell/2ul. 2ul aliquots of this resulting suspension were examined to determine the presence of a single cell before transfer to a well of a 96 well cluster dish. The clonal lines were maintained under conditions previously described for the parent line [5] i.e. at 37°C in a humidified air/CO2 (90:10) environment. Cells were initially maintained in conditioned Dulbecco’s modified Eagle’s medium (DMEM) supplemented with foetal calf serum (10% v/v) and 2mM L-glutamine in 75cm2 flasks. When the cell lines had become established basic supplemented DMEM was used instead of conditioned medium. Cells for assay were grown in 24 well cluster dishes. The clonal cells were fed after 48h and were passaged every 5 days (1:5 split ratio).

Confluent monolayers of clonal cells were loaded with [3H]-inositol for 24h prior to challenge with a range of agonists. Following assay, the accumulation of total [3H]-inositol phosphates in the disrupted cell monolayer was determined using anion exchange chromatography and liquid scintillation counting.

The relative sizes of the responses to stimulation by histamine (HA) and N-cyclopentyladenosine (CPA) varied markedly between the clonal lines (see Fig.1). For example the responses to CPA (100nM) were 2.0 ± 0.3, 6.2 ± 1.5 and 16.3 ± 2.5 fold over basal levels in the clonal lines designated TW1, TW3 and TW5 respectively. In the same cell lines the responses to HA (0.1mM) were respectively 3.4 ± 0.5, 5.8 ± 1.1 and 7.9 ± 1.1 fold over basal levels (n=6). The responses to other agonists were also variable e.g. giving values for noradrenaline (0.1mM) of 6.2 ± 1.6, 10.9 ± 2.0 and 16.5 ± 3.6 in the clonal lines TW1, TW2 and TW4 respectively.

Preliminary parallel studies have been carried out involving the measurement of intracellular calcium levels in these cells using the calcium-sensitive fluorescent dye fura-2 (Dickenson & Hill, data not shown).

To conclude, these preliminary agonist screens on the DDTMF-2 clonal cell lines confirm the heterogeneous nature of the parent cell line. Whether these variations in [3H]-inositol phosphate responses are a result of differing receptor numbers, or are due to differing agonist efficacy in the clonal lines is unknown at present.

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Figure 1. 3H-inositol phosphate accumulation in subclones of the DDTMF-2 cell line (n = 4 experiments).