ACTIVATION OF D1 AND D2 DOPAMINE RECEPTOR SUBTYPES INHIBIT CALCIUM CURRENT IN NG108-15 CELLS

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A number of molecular subtypes of dopamine (DA) receptors exist, though they are grouped in terms of their pharmacological and functional characteristics into D1 and D2 receptor families. Both D1 and D2 DA receptors can inhibit calcium (Ca^{2+}) current (Seabrook et al, 1994; Surmeier et al, 1995). We provide evidence for both types of modulation in the same cell and investigate the mechanisms underlying the Ca^{2+} current modulation. NG108-15 cells were differentiated using PGE_1 (10 μM) and IBMX (50 μM). Whole-cell patch clamp recordings of Ca^{2+} current were made (Axopatch 200A). Extracellular solution contained (in mM) 120 TEACl, 10 Hepes, 11.1 glucose, 3 KCl, 1.5 $MgCl_2$, 10 $CaCl_2$ (pH 7.4 with NaOH). The intracellular solution contained: 110 CsCl, 40 Hepes, 3 EGTA, 3 $MgCl_2$ (pH 7.4 with CsOH). For all recordings, the holding potential was −70mV. The effect of drugs on Ca^{2+} current was assessed during voltage steps to +20 mV, applied every 30s (pCLAMP, Axon Instruments). The effects of each drug treatment were measured after at least 90 s. The mean peak current during the three traces recorded before drug application was defined as 0% inhibition and all subsequent peak current measurements were normalised to this. All data are given as mean ± SEM.

Dopamine (20 μM) inhibited Ca^{2+} current (by 45 ± 12.2%, n = 7). This effect was characterized using agents selective for the D1 and D2 DA receptor families. Thus, Ca^{2+} current was depressed by the D1 agonist, SKF81297 (2 μM; 41.8 ± 4.9%, n = 18). This was prevented by the D1 antagonist, SCH 23390, at 0.1 μM (n = 9). D2 receptor-mediated modulation was also found. Thus, quinpirole (10 μM), inhibited Ca^{2+} current to a similar extent (41.5 ± 8.7%, n = 9) and this effect was prevented by the D2 antagonist, sulpiride (10 μM, n = 11). We have also investigated the mechanism underlying the receptor-Ca^{2+} channel coupling. First, we compared the effects of DA receptor agonists with noradrenaline (NA). Differentiated NG108-15 cells express α_2-adrenoreceptors. This receptor couples to calcium channels via a G protein βγ-subunit-dependent mechanism (Ikeda, 1996). NA (10 μM) in the presence of SKF81297 elicited much greater inhibition of Ca^{2+} current compared to prior exposure of the same cells to D1 receptor agonist alone (SKF81297 – 21.7 ± 3.1%; SKF81297/NA – 52.2 ± 6.1%, n = 7). However, NA co-applied with quinpirole showed a much smaller increase in current inhibition compared to the D2 receptor agonist alone (quinpirole – 43.7 ± 6.8%; quinpirole/NA – 54.5 ± 5.5%, n = 7). This may indicate that D1 agonist acts via a different mechanism (possibly including effects on different calcium channel subtypes) to that of D2 agonist and NA. We investigated the pathway activated by D1 agonist and found that the modulation of Ca^{2+} current appears to be mediated via indirect 2nd messenger signalling. Thus, the protein kinase C (PKC) inhibitor, chelerythine (5 μM) greatly reduced the effect of D1 agonist (n = 13), but the protein kinase A inhibitor (KT 5720, 0.3 μM) had no effect (n = 13). In summary, DA modulates Ca^{2+} current via both D1 and D2 receptors. The D1 receptor family couples to Ca^{2+} channels via an indirect pathway involving PKC-dependent signalling.

Ikeda (1996) *Nature* **380**:255-258.
Seabrook *et al.* (1994) *Br J Pharmacol* **111**:1061-1066.
Surmeier *et al.* (1995) *Neuron* **14**:385-397.