CAFFEINE INDUCED IONIC CURRENTS IN SINGLE RECTAL GLAND CELLS OF SQUALUS ACANTHIAS

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It has been shown that caffeine causes intracellular Ca^{2+} release from the sarcoplasmic reticulum of muscle cells (Chapman and Leoty, J. Physiol. Lond. 256:287-314, 1976; Konishi et al., J. Physiol. Lond. 355:605-618, 1984; Callewaert et al., Am. J. Physiol. 257:C147-152, 1989). A transient inward current has also been observed when single cardiac myocytes are briefly exposed to caffeine (Clusin et al., Am. J. Physiol. 245:H528-532, 1983; Mechmann and Pott, Nature Lond. 319:597-599, 1986). This current appears to be induced by intracellular Ca^{2+} release and is transported by Na^{+}-Ca^{2+} exchanger (Callewaert et al 1989). The effects of caffeine on secretory cells are less characterized. Here, we report that caffeine activates an inward or an outward current (depending on the holding potential) in freshly isolated single cells of dogfish rectal gland.

Single cells from freshly isolated (Xiao and Morad, Bulletin MDIBL, 1994) dogfish rectal gland were used in the present study. Caffeine activated currents were studied using whole-cell clamp technique (Hamill et al., Pflugers Arch. 391:85-100, 1981). The intracellular solution contained (in mM) 240 KCl or CsCl, 20 HEPES, 0.2 or 20 EGTA, 5 Mg_2ATP, 1 $MgCl_2$, 70 TMAO, 300 urea and pH was adjusted to 7.2 with KOH or CsOH; the bath solution contained (in mM) 270 NaCl, 250 urea, 5 $CaCl_2$, 5 or 0 KCl, 20 HEPES, 3 $MgCl_2$, 1 Glucose and pH was adjusted to 7.2 with NaOH. Rapid (< 50 ms) application of caffeine was accomplished by using a multi-barrelled concentration-clamp device. All experiments were carried out at room temperature (21-23°C).

Figure 1 illustrates the holding and caffeine activated currents in four separate cells from freshly isolated dogfish rectal glands. Lower panels of A and B show that when both the internal and external solutions contained K^{+}, the I-V relations appear to have significant rectification. 10 mM caffeine activated an outward current (upper panels) at a holding potential of -80 mV but an inward current when the holding potential was changed to -30 or 0 mV (middle panels). Panels C and D of Figure 1 illustrate that when the internal solution was changed to Cs^{+}, the application of caffeine induced only an inward current at the holding potential of -80 mV (upper panels), and failed to induce any current at the holding potential of 0 mV (middle panels). The current-voltage relation, under these conditions, is linear except for one point (lower panels). In Cs^{+} dialyzed cells bathed in 5 mM K^{+} an outward current was first observed when caffeine was applied immediately following patch formation and break, most likely because even though the pipette contained Cs^{+} complete dialysis had not taken place. In such cells, following complete Cs^{+} dialysis, rapid application of caffeine induced only an inward current.

Table 1 shows that more than 85 per cent of freshly isolated cells responded to caffeine. The average amplitude of currents for either inward or outward was about 200 pA. Time to the peak amplitude of currents was around 500 to 650 ms in freshly isolated gland cells.

The major finding in this study is that caffeine can activate an inward or an outward current in single cells of freshly isolated dogfish rectal gland. The direction of caffeine activating current was determined in part by the holding potential and the composition of intracellular solution. The caffeine-induced currents in the rectal gland cells were unlike those triggered by caffeine in muscle cells. Since only inward currents were found in the cell dialyzed with Cs^{+}, the outward current activated by caffeine at a holding potential of -80 mV appears to be related to K^{+} moving across the cell membrane. It is not clear as yet whether the outward current activated by caffeine is due to the activation of some kind of non-selective ionic channel or receptor-operated channel. It

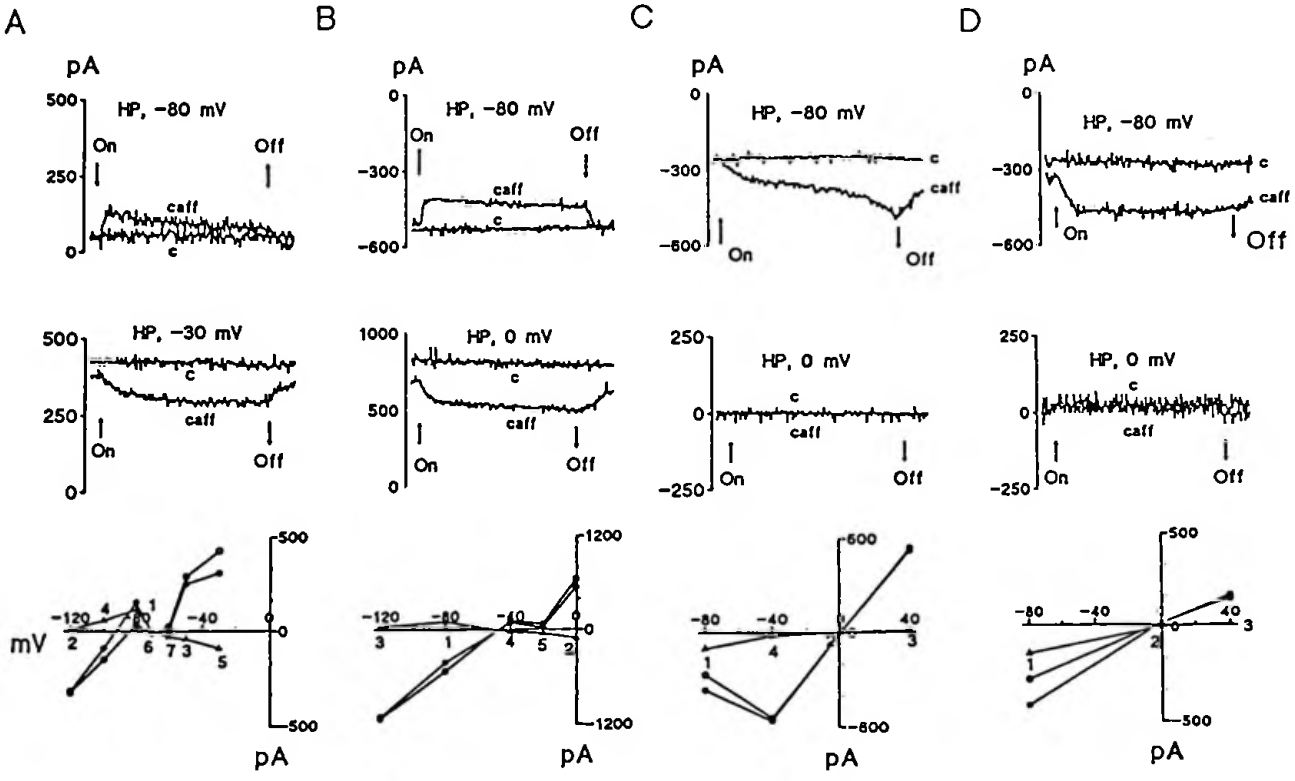

Figure 1. Caffeine activated inward or outward currents in single cells from freshly isolated dogfish rectal gland. A and B represent two cells in which both the internal and external solutions contained K^+. C and D are another two cells with internal solutions containing primarily Cs^+ instead of K^+. Upper panels, represent current tracing evoked by rapid application of 10 mM caffeine at a holding potential of -80 mV. Middle panels, currents were induced by rapid application of caffeine at a holding potential of -30 or 0 mV. On, represents the onset of puffing of caffeine; Off, the end of application of caffeine; c, control; caff, caffeine; HP, holding potential. Lower panels, show the voltage-dependence of caffeine-induced current at different holding potentials for control (O), 10 mM caffeine (●) and the difference current (▲) between caffeine and control conditions. The numbers in lower panels are the order of the application of caffeine. The pipette solution contained (in mM) 240 KCl or CsCl, 20 HEPES, 20 EGTA, 5 Mg_2ATP, 1 $MgCl_2$, 70 TMAO, 300 urea and pH was adjusted to 7.2 with KOH. The bath solution contained (in mM) 270 NaCl, 250 urea, 5 $CaCl_2$, 5 KCl, 20 HEPES, 3 $MgCl_2$, 1 Glucose and pH was adjusted to 7.2 with NaOH.

has been long known that compounds of similar structure to caffeine block the adenosine receptors. Since the onset of caffeine-induced current was slow (500 to 900 ms) the caffeine effect may be regulated by the activation of some kind of receptor which may in turn activate a K^+ conducting channel. This regulation may be restricted to a small range of membrane potential, because the maximum effect of the outward current activated by caffeine was at -80 mV. At less negative potentials (-30 or 0 mV) caffeine induced an inward current which may be carried by Na^+ or Cl^-. One possibility to consider is that caffeine by suppressing the phosphodiesterase activity, resulting in increased $[cAMP]_i$ may activate the 50 pS Cl^- channel (Greger et al., Pflugers Arch. 403:446-448, 1985).

Thus it appears that caffeine may activate a non-selective channel in single cells of dogfish rectal gland.

Table 1. Caffeine activated currents in freshly isolated versus cultured cells of dogfish rectal gland

Cell	n	Internal solution (mM)	External solution (mM)	HP (mV)	Caffeine response (%)	Direction and amplitude of current (pA)	Time to peak (ms)	C_m (pF)
Fresh	8	240 K^+	5 K^+	-80	75	outward 186±36	531±112	40±10
	3	240 Cs^+	5 K^+	-80	100	inward -203±28	616±186	29±1
	3	240 Cs^+	0 K^+	-80	100	inward -215±53	654±137	67±13
Cultured	14	240 Cs^+	0 K^+	-80	42	inward -414±75	varied	39±3

Current, time to peak and cell capacitance (C_m) are the mean ± S.E.M. with n being the number of cells treated with caffeine. HP, holding potential. Percentage of cells which responded to caffeine was calculated by number of responding cells divided by the total number of patches.

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