Nicotinamide–adenine dinucleotide regulates muscarinic receptor-coupled K^+ (M) channels in rodent NG108-15 cells

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1. The possible role of nicotinamide–adenine dinucleotide (NAD^+) and cyclic adenosine diphosphate ribose (cADPR) as regulators of M-type K^+ currents ($I_{K(M)}$) has been studied in whole-cell patch-clamped NG108-15 mouse neuroblastoma × rat glioma cells that had been transformed to express m1 muscarinic acetylcholine receptors (mAChRs).

2. Pre-incubation of NG108-15 cells for 6–8 h with streptozotocin (2–5 mM) reduced NAD^+ levels by 40–50%. Nicotinamide (2–5 mM) increased NAD^+ levels and prevented depletion by streptozotocin.

3. Streptozotocin pretreatment reduced the inhibition of $I_{K(M)}$ produced by 100 μM acetylcholine (ACh) from $51{\cdot}6 \pm 7{\cdot}0$ to $29{\cdot}1 \pm 7{\cdot}5$%. This was prevented by simultaneous pre-incubation with 2 mM nicotinamide or by adding 2 mM NAD^+ to the pipette solution. Neither procedure significantly affected the initial amplitude of $I_{K(M)}$.

4. Inclusion of 2 μM cADPR in the pipette solution induced a slow loss of $I_{K(M)}$ with a time constant of about 20 min.

5. It is concluded that mAChR-induced inhibition of $I_{K(M)}$ requires intracellular NAD^+. This might be needed for the formation of cADPR as a regulator or messenger for $I_{K(M)}$ inhibition.

Nicotinamide–adenine dinucleotide (NAD^+) is present in cells in millimolar concentrations. It functions as a co-enzyme and also serves as a substrate for adenosine diphosphate ribose (ADPR) synthetase or ADP-ribosyl-transferase (Kim, Jacobson & Jacobson, 1993). In addition, ADP-ribosyl-cyclase or NAD^+ glycohydrolase produces cyclic ADP-ribose (cADPR) or ADPR from NAD^+ (Kim *et al.* 1993). cADPR can release Ca^{2+} from intracellular Ca^{2+} stores and thus may subserve a cytoplasmic messenger function analogous to inositol trisphosphate (Galione, Lee & Busa, 1991; Takasawa, Nata, Yonekura & Okamoto, 1993; Galione, White, Willmott, Turner, Potter & Watson, 1993).

In the present experiments, we have used NG108-15 neuroblastoma × glioma hybrid cells (Higashida & Brown, 1986) to assess whether NAD^+ and/or cADPR might be involved in the regulation of neural ion channels, in particular the M-channel ($I_{K(M)}$). This is a voltage-gated K^+ channel which, in NG108–15 cells, is inhibited by bradykinin (Higashida & Brown, 1986) or in cells transformed to express m1 or m3 muscarinic acetylcholine receptors (mAChRs) by acetylcholine (ACh; Fukuda *et al.* 1988; Robbins, Marsh & Brown, 1993). Receptor-mediated inhibition of $I_{K(M)}$ appears to require a diffusible (presumably cytosolic) messenger (Selyanko, Stansfeld & Brown, 1992) but the identity of this messenger is not known.

We find that modification of intracellular NAD^+ levels does not affect $I_{K(M)}$ *per se* but alters the coupling between m1 mAChRs and $I_{K(M)}$. We also find that intracellular application of cADPR imitates the effect of ACh or of G protein activation in inhibiting $I_{K(M)}$, suggesting a potential role for the NAD^+–cADPR system in regulating or mediating $I_{K(M)}$ inhibition.

This manuscript was accepted as a Short Paper for rapid publication.

METHODS

Neuroblastoma × glioma NG108-15 hybrid cells transfected with porcine brain ACh muscarinic m1 receptor cDNA (subclone PM1-8 or PM1-27) were continuously grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS) and HAT (hypoxanthine, aminopterine and thymidine) in 10% CO_2, as described previously (Fukuda *et al.* 1988). Cells were cultured on polyornithine-coated 35 mm culture dishes (Higashida & Brown, 1986), and differentiated in DMEM supplemented with 1% FCS, hypoxanthine and thymidine with 0·25 mM dibutyryl cyclic AMP for 7 days. Streptozotocin and/or nicotinamide were added to the differentiation medium, and the differentiated cells were incubated further for the indicated periods. The medium was then discarded and cells washed once with 1 ml of ice-cold phosphate-buffered saline and rinsed with 1 ml of ice-cold 10 mM Hepes solution, pH 7·4, containing 50 mM nicotinamide. Cells were collected by scraping and sonicated in microtubes for 15 s. The cell homogenate was kept in boiling water for 5 min and then centrifuged at 15000 *g* for 5 min at 0 °C. The NAD^+ content in the supernatant of heat-inactivated cell homogenate was determined as described by Nisselbaum & Green (1969). The reaction mixture (1 ml) contained 0·08 mg Thiazolyl Blue (MTT), 0·3 mg phenazine methosulphate, 0·25 mg alcohol dehydrogenase, 0·065 M glycylglycine buffer at pH 7·4, 0·065 M nicotinamide, and 0·33 M ethanol with or without 0·1 ml of cell extract. The reactions were stopped after 10–12 min incubation by diluting an aliquot with five volumes of ice-cold water. The absorbance of the resulting solution was determined at 556 nm in a Shimazu DU spectrophotometer (Kyoto, Japan). Protein was determined by Bio-Rad Protein Assay kit (Hercules, CA, USA).

For electrophysiological recording, cells were transferred to 35 mm culture dishes, which had been precoated with polyornithine and grown in DMEM supplemented with 1% FCS, hypoxanthine, thymidine and 10 μM prostaglandin E_1 (PGE_1) and 0·5 mM 3-isobutyl-1-methylxanthine (IBMX) for 2–5 days (Robbins *et al.* 1993). The whole cell variant of the patch-clamp technique was used in discontinuous voltage-clamp mode (Axoclamp 2, Axon Instruments, Foster City, CA, USA), as previously described (Robbins *et al.* 1993). Cells were superfused with modified Krebs solution at 35 °C, composition (mM): NaCl, 120; KCl, 3; glucose, 11; $NaHCO_3$, 22·6; $MgCl_2$, 1·2; Hepes, 5; $CaCl_2$, 2·5; tetrodotoxin, 0·0005; pH 7·4, when gassed with 95% O_2 and 2–5% CO_2. Electrodes were normally filled with a solution containing (mM); potassium acetate, 90; KCl, 20; Hepes, 40; pH 7·4; $MgCl_2$, 3; EGTA, 3; $CaCl_2$, 1. The calculated free calcium concentration was 40 nM (Robbins *et al.* 1993). M-current deactivation tails were evoked by hyperpolarizing steps for 1 s to −50 or −60 mV. Voltage and current were recorded continuously on a Gould pen recorder (model 2400S). For bath application, control external solution with a flow rate between 5 and 10 ml min^{-1} was switched to 0·1 or 1 mM ACh dissolved in the external superfusate for the indicated period. Holding potentials were −20 or −30 mV.

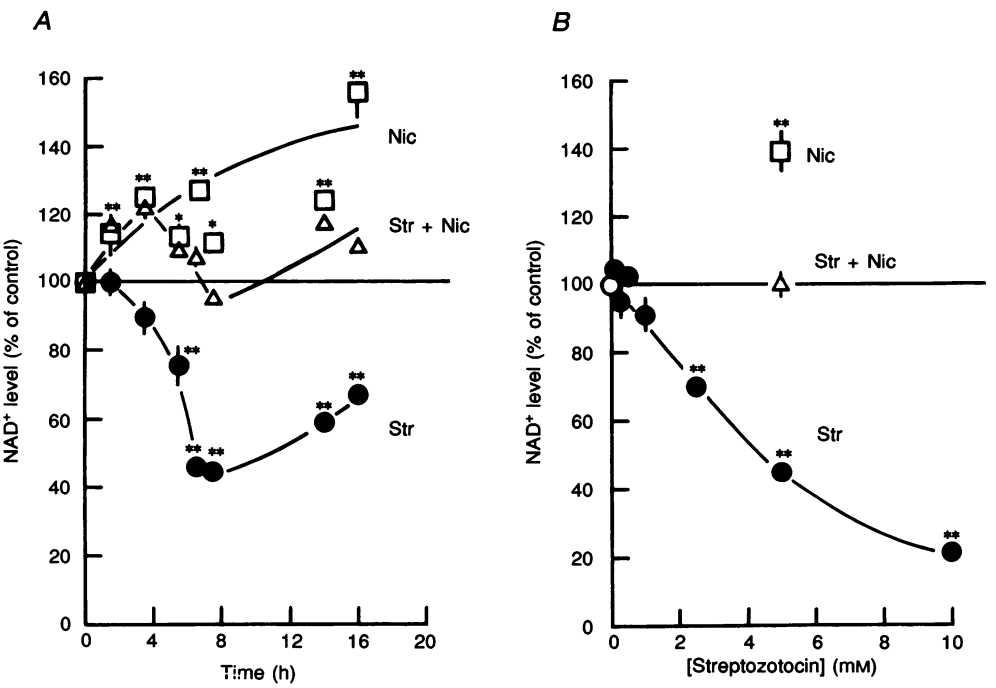

Figure 1. Time course and dose relationship of streptozotocin-induced NAD^+ degradation in m1-transformed NG108-15 cells

NAD^+ levels in cells incubated with streptozotocin (Str, ●) at 5 mM (*A*), or the indicated concentrations (*B*, abscissa), 5 mM nicotinamide (Nic, □) and 5 mM streptozotocin plus 5 mM nicotinamide (△) for the indicated periods (*A*, abscissa) or 7 h (*B*). Each point represents the mean value of 4 determinations in 2 independent experiments. Error bars shown in *A* and *B* indicate S.E.M. Control value for NAD^+ level in NGPM1-27 cells was 4·34 ± 0·70 nmol per 10^6 cells ($n = 7$). * or **, significantly different from control value at $P < 0·01$ or at $P < 0·001$, respectively.

RESULTS

In initial experiments we used the diabetogenic compound, streptozotocin, to modify NAD^+ levels. Streptozotocin has been reported previously to reduce NAD^+ levels in pancreatic β cells (Yamamoto, Uchigata & Okamoto, 1981) and liver cells (Schein & Loftus, 1968). In NG108-15 cells, 2–5 mM streptozotocin reduced NAD^+ levels by 40–50% after 6–8 h incubation (Fig. 1*A*). Levels then remained depressed for a further 10 h. This effect was dose dependent, with an IC_{50} of around 4 mM (Fig. 1*B*). In contrast, incubation with 2–5 mM nicotinamide increased NAD^+ levels by up to 30–50% and prevented the

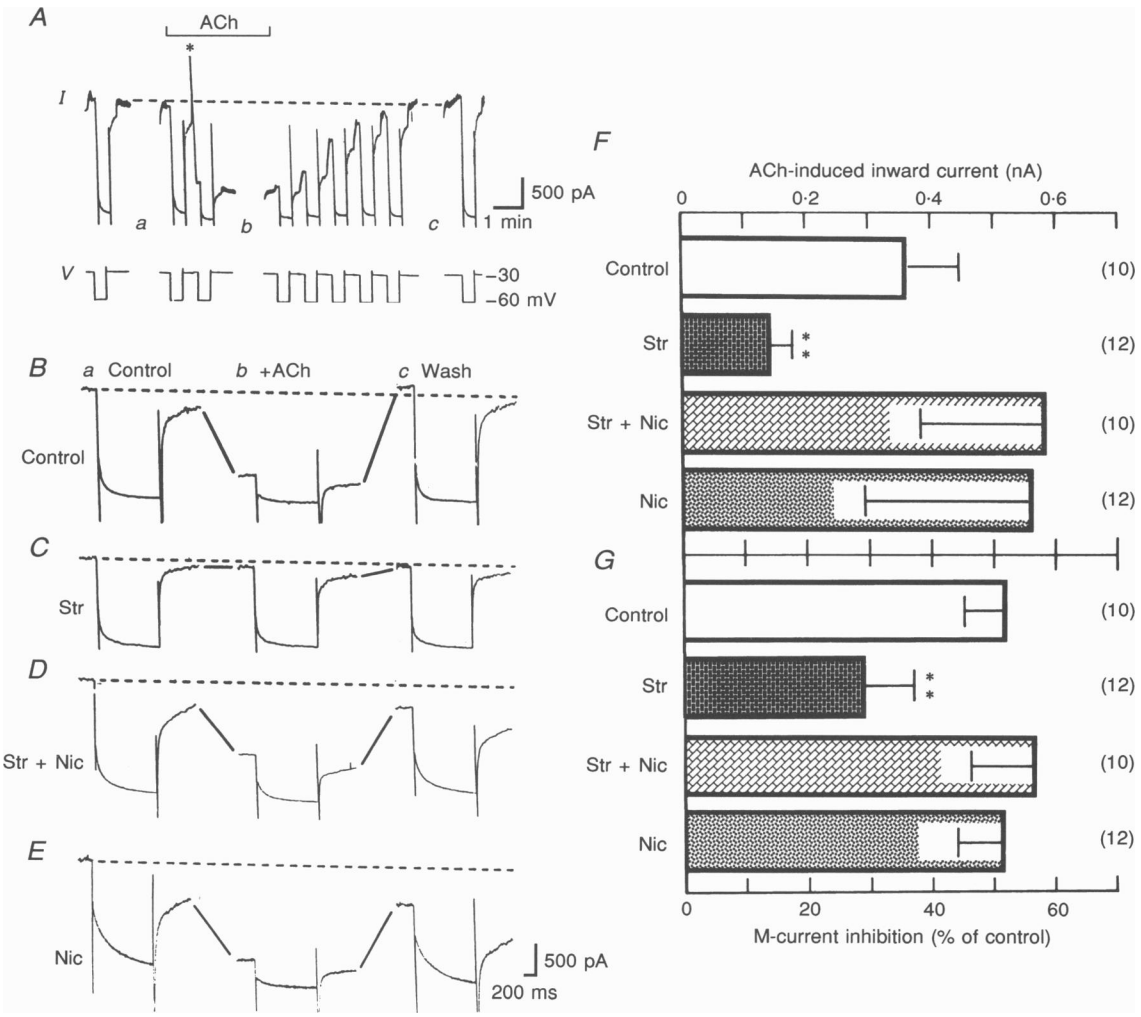

Figure 2. Acetylcholine-induced inward current and inhibition of M-current in m1-transformed NG108-15 cells treated with or without streptozotocin and/or nicotinamide

A, a typical result in a control NGPM1-8 cell. Uppermost, ACh (100 μM) was applied by bath perfusion for the duration indicated by the bar. Middle, current trace (*I*). The cell was held at −30 mV to activate $I_{K(M)}$ and stepped to −60 mV for 1 s every 30 s to deactivate $I_{K(M)}$ (*V*, shown in lower record). ACh produced a sustained inward current preceded in half of the cases by a transient outward current (indicated by asterisk,). Note that $I_{K(M)}$ was inhibited during the ACh-induced inward current and recovered on washing out ACh. *B–E*, four current traces evoked by voltage steps of −30 mV from holding potentials of −30 mV, as described in *A*. Currents were recorded before (*a*), during 100 μM ACh perfusion (*b*) and after washing out ACh (*c*) in NGPM1-8 cells. Records obtained: *B*, in an untreated control cell (Control); *C*, in a cell treated with 2 mM streptozotocin for 5–7 h (Str); *D*, in a cell treated with 2 mM streptozotocin and 2 mM nicotinamide simultaneously (Str + Nic); *E*, in a cell treated with 2 mM nicotinamide (Nic). The dashed line shows the initial current level from which ACh-induced inward currents were measured. Histograms on the right show the average amplitude of ACh-induced inward current (*F*) and amount of inhibition of $I_{K(M)}$ (*G*) recorded in cells under the various treatments as described in *B–E*. Number of cells tested indicated in parentheses. ** $P < 0{\cdot}01$ from control cells and $P < 0{\cdot}001$ from treated cells with streptozotocin and nicotinamide.

reduction of [NAD^+] by streptozotocin. These effects were slower in onset and required higher concentrations of streptozotocin than in β-cells but were probably mediated by a similar mechanism involving the formation of reactive intermediates leading to DNA strand breaks and stimulation of intracellular poly-ADPR synthetase (Okamoto, 1990), since in our experiments on NG108-15 cells we also detected increased nucleosomal DNA fragmentation after incubation for 6–17 h with 2–5 mM streptozotocin. However, in contrast to pancreatic β-cells (Yamamoto *et al.* 1981; Okamoto, 1990), no cell lysis was detectable after 15–18 h incubation with concentrations up to 5 mM, though higher concentrations (10–25 mM) produced clear evidence of cell damage.

We next tested the effects of streptozotocin on the inhibition of $I_{K(M)}$ in m1-transformed NG108-15 cells byacetylcholene (Fukuda *et al.* 1988; Robbins *et al.* 1993). In these experiments, $I_{K(M)}$ was activated by predepolarization to −30 or −20 mV, then deactivated by 1 s hyperpolarizing steps to −60 mV (see Fig. 2*A*). Application of 100 μM ACh produced an initial, transient outward current (due to activation of Ca^{2+}-dependent K^+ currents), followed by an inward current due to inhibition of $I_{K(M)}$ (Fukuda *et al.* 1988). During this inward current, $I_{K(M)}$ deactivation tail currents were inhibited by 51·6 ± 7·0% (mean ± S.E.M., $n = 10$; Fig. 2*A*, *B*, *F*, *G*). In cells pretreated with streptozotocin (2 mM for 7 h), ACh produced a smaller inward current with significantly less $I_{K(M)}$ inhibition (29·1 ± 7·5%, $n = 12$; 55% of the control value, $P < 0·01$; Fig. 2*C*, *F*, *G*). This effect of streptozotocin was completely prevented by the simultaneous incubation of the cells with 2 mM nicotinamide (Fig. 2*D*, *F*, *G*). Neither streptozotocin nor nicotinamide significantly affected the initial amplitude of $I_{K(M)}$ (Fig. 2*E*, *F*, *G*).

To test further whether the effect of streptozotocin resulted from the reduction in NAD^+ levels, we applied NAD^+ directly to the cell interior, by adding it to the patch-pipette solution (Fig. 3). Under the control condition (i.e. without NAD^+, but with 2 μM ATP and 0·5 mM GTP in the pipette solution), 100 μM ACh inhibited $I_{K(M)}$ by 57·3 ± 5·6% ($n = 6$). This was again significantly reduced to 23·5 ± 8·0% ($n = 8$; $P < 0·01$) after 6–8 h incubation with 2 mM streptozotocin. When 2 mM NAD^+ was included in the pipette solution, $I_{K(M)}$ inhibition by ACh was significantly *enhanced* to 85·5 ± 3·1% ($n = 6$, $P < 0·005$) and the inhibitory effect of streptozotocin was very substantially reduced, such that, after 2 mM streptozotocin, ACh still inhibited $I_{K(M)}$ by 67·3 ± 4·7% ($n = 7$). As before, neither streptozotocin nor application of intrapipette NAD^+ significantly affected the absolute amplitude of $I_{K(M)}$. (Mean amplitudes ± S.E.M. (pA): control, 331 ± 49; NAD^+, 579 ± 109; streptozotocin, 393 ± 44; NAD^+ + streptozotocin, 482 ± 96.)

In addition to inhibiting $I_{K(M)}$, activation of m1 mAChRs in NG108-15 cells increases inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) production (Fukuda *et al.* 1988), and (as noted above) the consequent release of intracellular Ca^{2+} frequently induces a transient Ca^{2+}-activated K^+ current preceding $I_{K(M)}$ inhibition (Fukuda *et al.* 1988; Neher, Marty, Fukuda, Kubo & Numa, 1988; Robbins *et al.* 1993). However, in contrast to its effect on M-current inhibition, streptozotocin pretreatment had no significant effect on either of the following three phospholipase C-driven

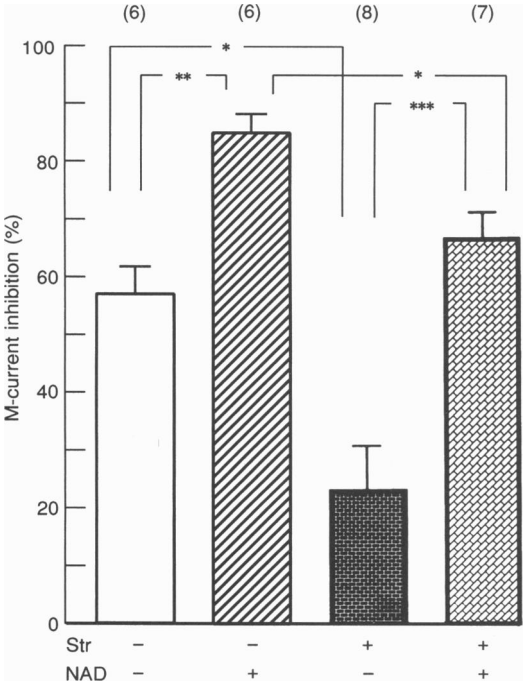

Figure 3. Effect on ACh-induced inhibition of $I_{K(M)}$ with intracellular application of NAD^+ into m1-transformed cells treated or untreated with streptozotocin

NGPM1-8 cells were differentiated with 10 μM PGE_1 and 0·5 mM IBMX for 3 days. The cells were further treated or untreated with 2 mM streptozotocin for 7 h. $I_{K(M)}$ was recorded using the patch solution described in Fig. 2, supplemented with 2 mM NAD^+, as indicated. ACh (100 μM) was superfused to induce $I_{K(M)}$ inhibition. Average inhibition of $I_{K(M)}$ amplitude was calculated from the number of cells indicated in parentheses. Str + or − indicates cells with or without treatment of streptozotocin. NAD^+ + or − indicates cells recorded with a patch pipette with or without NAD^+ in the recording solution. Error bars indicate S.E.M. * $P < 0·01$; ** $P < 0·005$; *** $P < 0·001$.

responses. Firstly, peak levels of Ins(1,4,5)P_3 at 10 s after application of 100 μM ACh to subclone NGPM1-8 of m1-transformed cells without or with 6 h pretreatment with 5 mM streptozotocin were 34·5 ± 4·3 and 35·1 ± 7·4 pmol per 10^6 cells, respectively ($n = 3$ in each case). Corresponding values for a subclone NGPM1-27 cell were 44·2 ± 3·9 and 39·0 ± 3·2 pmol per 10^6 cells ($n = 3$). Likewise, streptozotocin had no effect on resting levels of Ins(1,4,5)P_3, with mean values which ranged between 10·1 and 12·4 pmol per 10^6 cells. Secondly, the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) increased, in response to 100 μM ACh, to the same level in both non-treated- and streptozotocin- (5 mM, 6 h) treated NGPM1-8 cells ($n = 5$ in both cases), as measured by fura-2 emission (Kimura & Higashida, 1992). The peak $[Ca^{2+}]_i$ level was 482 ± 21% of the prestimulation level (110 ± 17 nM) in non-treated cells, whereas it was 477 ± 30% of the control level (96 ± 18 nM) in treated cells. The average half-decay time from the peak in $[Ca^{2+}]_i$ was 12·8 ± 2·4 s ($n = 5$) in untreated cells and 19·0 ± 3·2 s ($n = 5$) in streptozotocin-treated cells. Thirdly, the frequency of occurrence (16 out of 32 cells) and mean amplitude (0·43 ± 0·14 nA, $n = 6$) of the initial, transient outward current induced by ACh in streptozotocin-treated cells did not significantly differ from untreated cells (6 out of 12 cells; amplitude, 0·32 ± 0·13 nA, $n = 6$) or cells treated with streptozotocin plus nicotinamide (7 out of 16 cells; amplitude, 0·58 ± 0·36 nA, $n = 7$).

The effects of streptozotocin also appeared to be directed selectively at the transduction process following m1-receptor stimulation, since it did not affect responses to stimulating the endogenous m4 receptors. One such response is an inhibition of adenylate cyclase (Fukuda *et al.*

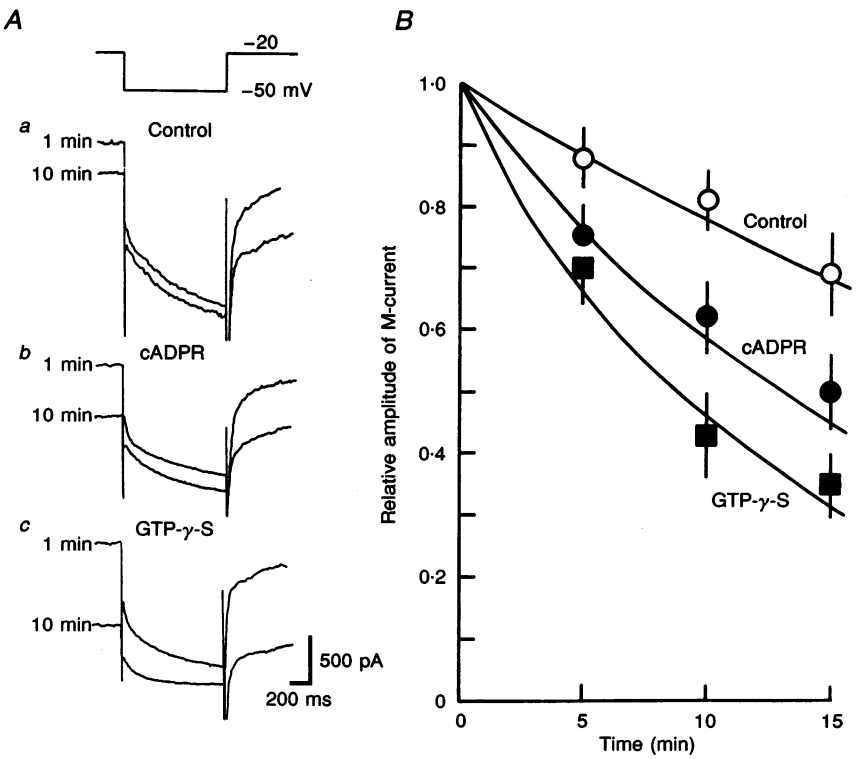

Figure 4. Effect of cADPR and GTP-γ-S on M-current

A, uppermost, voltage step to −50 mV for 1 s from a holding potential of −20 mV. Superimposed current records show deactivation of $I_{K(M)}$ on the same amplitude by stepping to −50 mV. Each pair of records shows currents observed 1 and 10 min after breaking the membrane under the patch pipettes after tight seal formation. Pipettes contained the control recording medium (Control) or medium supplemented with 2 μM cADPR or 0·5 mM GTP-γ-S. The graph (*B*) shows the change in $I_{K(M)}$ amplitude with time starting 1 min after breakthrough. (20–30 s was required to optimize the voltage-clamp amplifier.) The amplitude of the total current at the end of step pulses recorded at 5, 10, and 15 min after breakthrough is expressed as a percentage of that at 1 min. Values are the means ± S.E.M. of 3–5 cells. Curves are drawn according to the expression:

$$I_t = I_0 \exp(-k\,t),$$

where I_t and I_0 represent current amplitude at times t and 0 min respectively and k is a constant. Values for k (mean min^{-1} ± S.E.M., pooled data) were: controls, −0·025 ± 0·0008; cADPR, −0·054 ± 0·0007; GTP-γ-S, −0·080 ± 0·0013. Values for cADPR and GTP-γ-S were significantly greater than controls ($P < 0·0001$).

1988). Although streptozotocin (5 mM; 4–7 h) reduced basal cyclic AMP levels (from 399 ± 46 to 191 ± 82 pmol (mg protein)$^{-1}$, $n = 4$), it affected neither the increase in cyclic AMP produced by 10 min incubation with 10 μM PGE_1 (control, 2793 ± 147; with streptozotocin, 2397 ± 192 pmol (mg protein)$^{-1}$, $n = 4$), nor the inhibition of this PGE_1-stimulated increase by 10 μM carbachol (60 and 61% without and with streptozotocin, respectively). Streptozotocin did not significantly reduce the 42% inhibition of PGE_1-stimulated adenylate cyclase by carbachol in cells transfected to express exogenous m2 receptors (Fukuda *et al.* 1988).

Finally, effects of streptozotocin on $I_{K(M)}$ inhibition did not appear to result from a more general toxic action, since the concentrations required were below those which produced visible cell damage and cells exhibited normal action potentials, inward Na^+ currents, low- and high-threshold Ca^{2+} currents or delayed rectifier K^+ currents, as well as normal-amplitude K^+ (M) currents as noted above.

Hence, these experiments suggest that there is a specific requirement for NAD^+ in the transduction process leading from m1 mAChR stimulation to $I_{K(M)}$ inhibition. What is the nature of this requirement? One possibility is that it might be required for the formation of cyclic ADP-ribose (cADPR), implying that cADPR might act as one of the messengers responsible for $I_{K(M)}$ inhibition. We therefore tested whether intracellular application of cADPR could inhibit $I_{K(M)}$. Addition of 0·2–2·0 μM cADPR to the patch-pipette solution produced a slowly developing, partial inhibition (55%) of $I_{K(M)}$, of similar time course (though lesser magnitude) to that produced by adding the G protein activator GTP-γ-S (Robbins *et al.* 1993; Fig. 4). Further support was obtained by recording the time course of $I_{K(M)}$ inhibition using 2 μM cADPR inactivated by ultraviolet light exposure (for 60 min) in a second set of experiments: the average decay constant ($-0{\cdot}014 \pm 0{\cdot}0008$ min^{-1}, $n = 21$) by inactivated cADPR was the same as the control value ($-0{\cdot}014 \pm 0{\cdot}001$ min^{-1}, $n = 18$), but was significantly different from the decay constants using intact cADPR ($-0{\cdot}030 \pm 0{\cdot}0009$ min^{-1}, $n = 25$) at $P < 0{\cdot}001$ and GTP-γ-S ($-0{\cdot}089 \pm 0{\cdot}0051$ min^{-1}, $n = 20$) at $P < 0{\cdot}001$ (see also legend to Fig. 4).

'Breakthrough' with pipettes filled with 200 nM cADPR increased $[Ca^{2+}]_i$ to 780 ± 39 nM ($n = 5$) from the basal level of 120 ± 14 nM ($n = 4$). This is in accord with the Ca^{2+}-releasing effect of cADPR previously reported in neuronal cells (White, Watson & Galione, 1993; Hua *et al.* 1994). However, this effect may not have been responsible for the inhibition of $I_{K(M)}$ since comparable inhibition of $I_{K(M)}$ by intrapipette cADPR (200 or 400 nM) was observed using either 3 or 25 mM EGTA in the pipette solution, or on adding 10 μM BAPTA AM to the bathing solution (Kimura & Higashida, 1992), which prevented the rise in $[Ca^{2+}]_i$ induced by cADPR. The $[Ca^{2+}]_i$ increased by 178 ± 29 nM ($n = 8$) in the presence of 200 nM cADPR together with 3 mM EGTA from the pre-injection level of 78 ± 5·3 nM ($n = 8$) and by 70 ± 12 nM ($n = 3$) from 33 ± 2·7 nM ($n = 3$) with 25 mM EGTA.

DISCUSSION

The principal point established in these experiments is that adequate levels of intracellular NAD^+ appear to be an essential requirement for maintaining mAChR-mediated inhibition of $I_{K(M)}$, since reduction of NAD^+ levels was associated with reduced coupling efficiency and this was overcome by restoring NAD^+ levels (either indirectly with nicotinamide or directly by adding NAD^+ to the intracellular solution). This was not accompanied by any obvious change in $I_{K(M)}$ itself, nor in other membrane currents inspected. Further, changes in NAD^+ did not affect the inhibition of adenylate cyclase produced by stimulating the endogenous m4 or exogenous m2 mAChRs, suggesting that NAD^+ is specifically required for M-current inhibition.

One possibility is that NAD^+ might be needed for ADP ribosylation of associated proteins, including G proteins in NG108-15 cells (Donnelly, Boyd & MacDermot, 1992). Indeed, there is some evidence to suggest that an associated G protein may be necessary to maintain certain aspects of M-channel function (Stansfeld, Marsh, Gibb & Brown, 1993). An argument against this, however, is that the effect of free nicotinamide *resembled* that of NAD^+ instead of opposing it (as would be expected for NAD^+-driven ADP ribosylation). Further, changes in NAD^+ did not affect m1 mAChR-induced activation of phospholipase C or the subsequent Ca^{2+}-activated K^+ current, which are likely to be mediated by the same G protein (G_q) as that mediating the inhibition of $I_{K(M)}$ (Wilk-Blaszczak, Gutowski, Sternweis & Belardetti, 1994; cf. Caulfield *et al.* 1994; S. Jones & M. P. Caulfield, unpublished observations). Hence, it seems more likely that the requirement for NAD^+ lies downstream of both receptors and G protein activation.

An alternative, and attractive, possibility is that NAD^+ is necessary for the synthesis of cADPR, since our experiments show that cADPR is itself capable of reducing $I_{K(M)}$ when added to the cell interior. This seems unlikely to have been due to the release of Ca^{2+} since 25 mM EGTA did not prevent $I_{K(M)}$ inhibition by cADPR, even though it reduced the peak value of $[Ca^{2+}]_i$ following intracellular application of cADPR to no more than the resting level observed using 3 mM EGTA. Further, there was no difference in the ACh-induced rise in $[Ca^{2+}]_i$ between streptozotocin-treated and control cells. This is in accord with previous experiments suggesting that the initial rise in $[Ca^{2+}]_i$ produced by mAChR stimulation results overwhelmingly from the effect of $InsP_3$ (Robbins *et al.* 1993), rather than from the action of cADPR, and further reinforces the idea that the rise in Ca^{2+} itself is not likely to be the direct cause of $I_{K(M)}$ inhibition. Instead, cADPR

might be involved in some other manner in the transduction process leading to $I_{K(M)}$ inhibition. Further tests using cADPR antagonists may illuminate this possibility.

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Acknowledgements

This work was supported by grants from the Japanese Ministry of Education, Science and Culture and the UK Medical Research Council.

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Received 31 August 1994; accepted 7 November 1994.