FEBS Letters 379 (1996) 236–238 FEBS 16587

Streptozotocin, an inducer of NAD^+ decrease, attenuates M-potassium current inhibition by ATP, bradykinin, angiotensin II, endothelin 1 and acetylcholine in NG108–15 cells

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Received 29 November 1995; revised version received 18 December 1995

Abstract The M-potassium current was inhibited by bath application of 100 μM ATP, 10 nM bradykinin, 100 nM angiotensin II and 100 nM endothelin 1 as well as by 10 μM acetylcholine in an m1-muscarinic acetylcholine receptor-transformed NG108–15 cell line. The inhibition of M-current was attenuated in cells pretreated with 5 mM streptozotocin for 5–15 h and restored by simultaneous incubation with 5 mM nicotinamide. The results suggest that signal transduction from these five different receptors to M channels shares a common pathway which is susceptible to a streptozotocin-induced decrease in cellular NAD^+ content.

Key words: K^+ current; Signal transduction; Second messenger; NAD^+; Neuroblastoma × glioma hybrid cell

1. Introduction

The voltage-dependent potassium M-current leads to up-modulation of the membrane excitability of neuronal cells, when inhibited by muscarinic agonists [1]. The M-current inhibition is elicited by a number of different transmitters, including bradykinin [10], ATP, UTP [7], angiotensin II [19], endothelin 1 [15], substance P and LHRH [13]. However, the signal transduction pathway from these receptors to M channels is not yet clear [5,18]. Recently, we have shown that the muscarinic acetylcholine receptor (mAChR)-M channel coupling is partially blocked in streptozotocin-treated NG108–15 hybrid cells in which the cellular NAD^+ concentration is reduced, and that the M-current is slowly decreased by intracellular injection of cADP ribose (cADPR), a product of NAD^+ formed by ADP ribosyl cyclase [11]. Therefore, we have proposed that NAD^+ or NAD^+ metabolites may be involved in the signal pathway from mAChRs to M channels [11]. Here, we present data showing that the M-current inhibition induced by five different agonists is blocked to the same extent in streptozotocin-treated NG108–15 cells, supporting this hypothesis.

2. Materials and methods

2.1. Cell culture

NGPM1–27 cells, a subclone of m1-mAChR-transformed NG108–15 cells [9], were used. Cells were maintained in culture and differentiated with 0.25 mM dibutyryl cAMP for 12–21 days as described previously [10]. 5 mM streptozotocin and/or nicotinamide were added in serum-containing Dulbecco's modified Eagle's medium (DMEM), in which the cells were incubated for 5–15 h before electrophysiological recordings [11].

2.2. Electrophysiological recordings

Currents were measured at 35°C by the whole-cell patch-clamp method as described previously [11]. Patch electrodes were filled with a 90 mM K-citrate solution whose composition is given by Robbins et al. [18]. The electrode resistance was about 2–5 MΩ. Cells were superfused with 10 mM Hepes-buffered DMEM with or without agonists. The method of measuring M-current was described previously [11]. Some of the data was processed by p-Clamp.

2.3. Materials

Bradykinin, angiotensin II and human endothelin 1 were purchased from Peptide Research (Osaka, Japan). ATP and ACh were purchased from Sigma (St Louis, MO).

3. Results

Bath perfusion with the maximum dose of five different agonists (100 μM ATP, 10 nM bradykinin, 100 nM angiotensin II, 100 nM endothelin 1 and 10 μM ACh) of NGPM1–27 cells, voltage-clamped at a depolarized membrane potential of −20 mV, produced a steady inward current ranging from 0.2 to 1.5 nA as previously described in this cell line [9,11,18] and in the parental NG108–15 cells [7,10,15]. Fig. 1A shows the time course of ATP-induced current changes as a representative example of all the agonist-induced inward current. These inward currents were associated with an inhibition of the M-current in response to a hyperpolarizing step as shown in five cases of ATP (Fig. 1B), bradykinin (Fig. 2A), angiotensin II (Fig. 2B), endothelin 1 (Fig. 2C) and ACh (Fig. 2D). The amplitude of the M-current inhibition (calculated from $(x-y)/x \times 100$, where x and y are shown in Fig. 1C) was on average 41 ± 3.1% (mean ± S.E.M., $n = 14$) for ATP, 43 ± 6.1% ($n = 9$) for bradykinin, 37 ± 3.6% ($n = 11$) for angiotensin II, 37 ± 5.3% ($n = 5$) for endothelin 1 and 57 ± 4.8% ($n = 6$) for ACh, respectively (Table 1).

The same set of agonists were applied to NGPM1–27 cells pretreated with 5 mM streptozotocin for 5–15 h. The inward current and associated M current inhibition induced by the five agonists were attenuated significantly ($P < 0.05$ or < 0.01, Table 1) as shown in Fig. 3A–E. The mean inhibition of M-current was reduced to 22% of the control response for ATP, 44% for bradykinin, 19% for angiotensin II, 41% for endothelin 1 and 30% for ACh, respectively (streptozotocin/control × 100 in Table 1). As expected, this inhibitory effect of streptozotocin on the agonist-induced M-current inhibition was restored by simultaneous incubation with 5 mM nicotinamide. 5 mM nicotinamide alone had a small but negligible effect (Table 1).

4. Discussion

The results show that functional P_{2u} ATP [7,12,14,16] B_2

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SSDI 0014-5793(95)01516-7

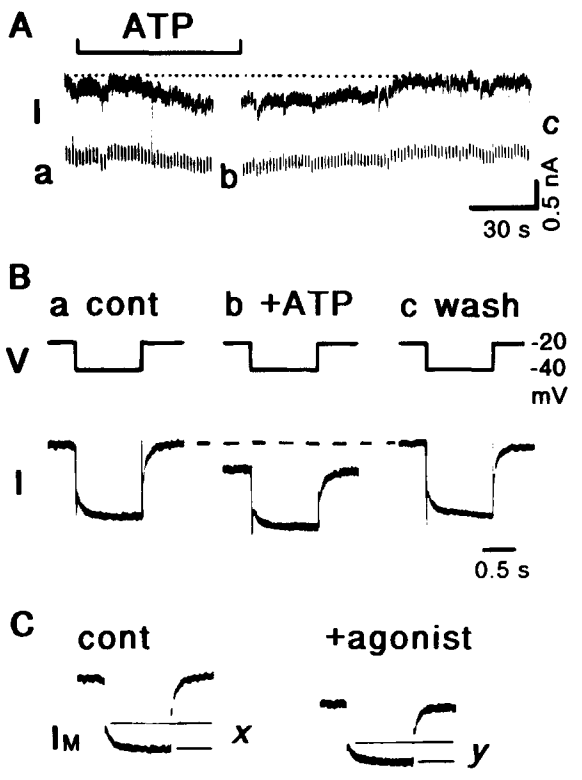

Fig. 1. ATP-induced inward current and M-current inhibition in an m1-transformed NGPM1–27 cell. The untreated control cell was held at −20 mV to activate M-current and stepped to −40 mV for 1 s every 30 s to deactivate it. (A) ATP (100 μM) was applied by bath perfusion for the duration indicated by the bar. ATP produced a sustained inward current (I). Repetitive inward transients were evoked by such voltage steps. The dashed line shows the initial current level from which bath application of ATP induced an inward (downward) shift of holding current. (B) Expanded records of voltage steps (V) and current transients (I). The currents were recorded before (a), during perfusion with 100 μM ATP (b) and after washing out it (c) at the indicated times in (A). (C) The diagram shows the measured M-current (I_M) in two current traces obtained before (x) and after (y) agonist perfusion.

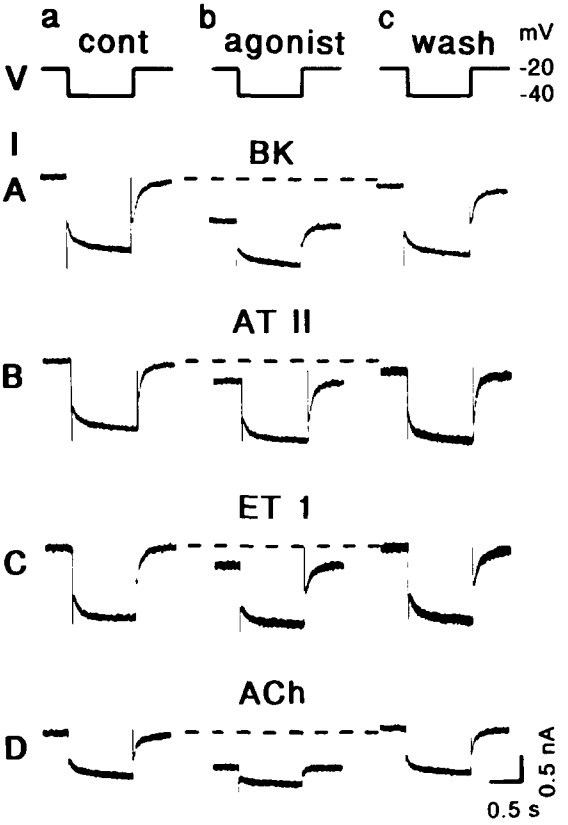

Fig. 2. Four different agonists-induced inward current and inhibition of M-current in an untreated control NGPM1–27 cell. The same cell shown in Fig. 1 was held at −20 mV to activate M-current and stepped to −40 mV for 1 s every 30 s to deactivate it. Current traces evoked by such voltage steps are shown. Currents were obtained before (a), during perfusion with each agonist (b) and after washing out them (c). Agonists used are 10 nM bradykinin (BK, A), 100 nM angiotnsin II (AT II, B), 100 nM endothelin 1 (ET 1, C), and 10 μM ACh (D).

bradykinin [10,17,20,21], AT_2 angiotensin [2–4] and endothelin [6,8,15,22] receptors, which are expressed in parental NG108–15 cells, are present in NG108–15 cells overexpressing m1 mAChRs and that they elicit an essentially equal amount of the M-current inhibition. Furthermore, the results suggest that the signal pathways from these five receptors, which are originally reported to couple to phospholipase C [4,6,8,12,14,15,17,20,21], to M channels merge into one, because M-current inhibition was susceptible to streptozotocin treatment and was restored by an addition of nicotinamide. Our previous measurement shows that in streptozotocin-treated NGPM1–27 cells, cellular NAD^+ content is reduced to about 40–50% of the control level

Table 1
Agonist-induced M-current inhibition in NGPM1–27 cells under various conditions

	Percentage inhibition of M-current (n)			
Agonist	Control	Streptozotocin	Streptozotocin + Nicotinamide	Nicotinamide
ATP	41 ± 3.1 (14)	9.1 ± 5.5** (10)	33 ± 5.6 (5)	51 ± 5.5 (4)
Bradykinin	43 ± 6.1 (9)	19 ± 6.2* (6)	38 ± 7.2 (4)	50 ± 8.4 (4)
Angiotensin II	37 ± 3.6 (11)	7.0 ± 4.7** (4)	52 ± 2.8 (3)	41 ± 4.2 (3)
Endothelin 1	37 ± 5.3 (5)	15 ± 4.6* (8)	27 ± 4.0 (4)	38 ± 4.5 (5)
ACh	57 ± 4.8 (6)	17 ± 9.8* (5)	46 ± 9.0 (5)	48 ± 6.1 (4)

M-current was recorded as described in Fig. 1. Mean percentage inhibition of M-current was calculated from $(x-y)/x \times 100$, where the amplitude of M-current before (x in Fig. 1C) and after (y in Fig. 1C) bath application of 100 μM ATP, 10 nM bradykinin, 100 nM angiotensin II, 100 nM endothelin 1 and 10 μM ACh. Currents were recorded in untreated control cells or cells treated for 5–15 h with 5 mM streptozotocin, with 5 mM streptozotocin+5 mM nicotinamide and with 5 mM nicotinamide, respectively. Values are the mean ± S.E.M. Number of cells tested is indicated in parentheses. *$P < 0.05$ and **$P < 0.01$ from control cells.

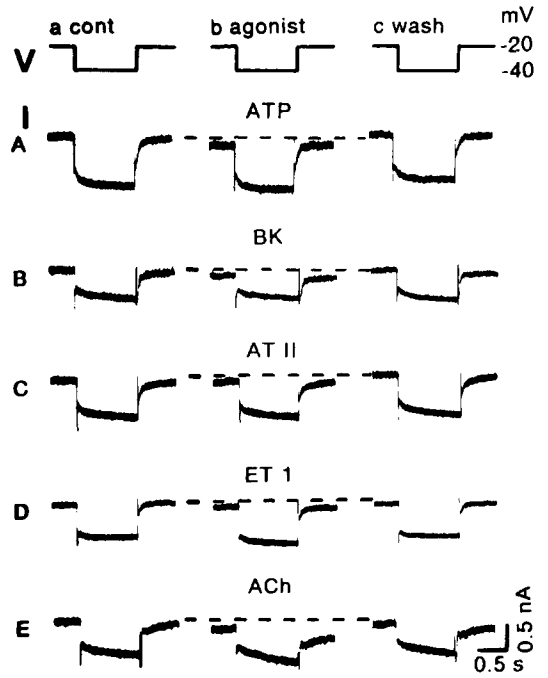

Fig. 3. Five different agonists-induced inward current and inhibition of M-current in streptozotocin-treated NGPM1–27 cells. Currents were recorded as shown in Figs. 1 and 2 in 5 different cells treated with 5 mM streptozotocin for 5–15 h. Currents were obtained before (a), during perfusion (b) with 100 μM ATP (A), 10 nM bradykinin (BK, B), 100 nM angiotnsin II (AT II, C), 100 nM endothelin 1 (ET 1, D) and 10 μM ACh (E) and after washing out them (c).

[11] and that this level is recovered by simultaneous addition of nicotinamide, a precursor of NAD^+. Thus, it is likely that the partial blockade of M-current inhibition induced by the four different agonists is closely related to the cellular NAD^+ level as suggested for m1 mAChRs.

It is likely that slow M-current inhibition is mediated by a diffusible second messenger(s) [5,9,10,19]. As has been proposed, suppression of M-current by angiotensin II seems to share a common signalling pathway with suppression by muscarinic agonists [19]. Furthermore, cADPR, a NAD^+ metabolite, also inhibited M-current in NG108–15 cells [11]. In line with this idea, we propose that these five receptors may share a common pathway in this cell line. Although it is not yet clear, it is possible that cADPR is a common mediator in their downstream pathway.

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