Cannabinoid receptor CB1 activates the Na^+/H^+ exchanger NHE-1 isoform via G_i-mediated mitogen activated protein kinase signaling transduction pathways

M. Bouaboula[a], L. Bianchini[b], F.R. McKenzie[b], J. Pouyssegur[b], P. Casellas[a,*]

[a] *Sanofi Recherche, Ligne Immunologie, 371 rue du Prof. Joseph Blayac, F-34184 Montpellier Cedex 4, France*
[b] *UMR CNRS 6543, Centre de Biochimie, Faculté des Sciences, Parc Valrose, F-06108 Nice Cedex 2, France*

Received 5 March 1999

Abstract We previously showed that the cannabinoid receptor CB1 stably transfected in Chinese hamster ovary cells was constitutively active and could be inhibited by the inverse agonist SR 141716A. In the present study, we demonstrate that the cannabinoid agonist CP-55940 induced cytosol alkalinization of CHO-CB1 cells in a dose- and time-dependent manner via activation of the Na^+/H^+ exchanger NHE-1 isoform. By contrast, the inverse agonist SR 141716A induced acidification of the cell cytosol, suggesting that the Na^+/H^+ exchanger NHE-1 was constitutively activated by the CB1 receptor. CB1-mediated NHE1 activation was prevented by both pertussis toxin treatment and the specific MAP kinase inhibitor PD98059. NHE-1 and p42/p44 MAPK had a similar time course of activation in response to the addition of CP-55940 to CHO-CB1 cells. These results suggest that CB1 stimulates NHE-1 by $G_{i/o}$-mediated activation of p42/p44 MAP kinase and highlight a cellular physiological process targeted by CB1.

Key words: Cannabinoid receptor 1; SR 141716A; Inverse agonist; Na^+/H^+ exchanger NHE-1 isoform; Mitogen-activated protein kinase

1. Introduction

Despite the broad physiological effects described for Δ^9-tetrahydrocannabinol (Δ^9-THC), the main active substance of marijuana, its mechanism of action only began being described a few years ago following the identification and cloning of the specific cannabinoid receptor. The central cannabinoid receptor (CB1) was cloned from both rats and humans [1,2] and shown to be expressed primarily in brain tissues [3,4]. CB1 mRNA has also been found to a lesser extent in testis [3], spleen [5] and leukocytes [6]. A second cannabinoid receptor (CB2) was cloned and recently characterized. CB2 is expressed in macrophages from the marginal zone of spleen, in B lymphocytes and NK cells, but not in brain tissues [7,8]. Both CB1 and CB2 receptors belong to the G-protein-coupled receptor (GPCR) superfamily.

It is now clearly established that Δ^9-THC and other potent synthetic cannabinoid receptor agonists, as well as anandamide and 2-arachidonylglycerol, the putative endogenous ligands [9–11], bind specifically to cannabinoid receptors.

CB1 and CB2 stimulation induces several biological responses, including inhibition of adenylyl cyclase (AC) [12–14], activation of mitogen-activated protein kinases (MAPK) [15,16], induction of the immediate-early gene (IEG) Krox 24 in vitro [16,17] and also in vivo [18,19]. CB1, but not CB2, was shown to induce ion channel modulation [20,21]. These cannabinoid effects are mediated by a pertussis toxin (PTX)-sensitive guanine nucleotide-binding protein (G_i protein). The CB1 coupled cellular responses were selectively prevented by the specific potent CB1 antagonist SR 141716A [22,23].

We recently showed that SR 141716A displayed a negative intrinsic activity towards the autoactivated CB1 transfected in Chinese hamster ovary (CHO) cells and that this inverse agonist was able to negatively control signal transduction mediated by G_i-dependent receptor tyrosine kinases like insulin or IGF1 [24].

Na^+/H^+ exchangers (NHE) are key electroneutral transmembrane transporters involved in multiple cellular functions, such as intracellular pH regulation, control of cell volume and transepithelial ion transport. To date five distinct isoforms of NHE, which differ in their kinetics and pharmacological properties, have been identified in mammalian cells (review in [25]). They are differentially expressed in various tissues, suggesting that the individual isoforms have distinct functions.

NHE-1, the first Na^+/H^+ exchanger isoform to be cloned [26], is ubiquitously expressed and appears to be the predominant species in non-epithelial cells where it has been shown to play a major role in pH_i homeostasis and cell volume regulation [27]. NHE-2 and NHE-3 are prominent in intestinal and renal tissues where they take part in transepithelial NaCl transport. The two other isoforms NHE-4 and NHE-5 have not been fully characterized.

NHE-1 activity can be modulated by a remarkably wide variety of stimuli, including growth factors, cytokines, tumor promoters, hormones and physical factors such as changes in cell volumes, or cell spreading [27]. Extracellular stimuli were shown to activate NHE-1 by increasing its sensitivity to intracellular H^+, resulting in cytoplasmic alkalinization [28–30]. The mechanism underlying this shift in pH_i sensitivity has not yet been fully elucidated. The p42/p44 MAPK cascade was recently shown to play a major role in the activation of NHE-1 by growth factors [31] or hormones such as arginine vasopressin [32].

The experiments described in this article were conducted to explore the cannabinoid receptor CB1 induction effect on

*Corresponding author. Fax: (33) 4 67 10 60 00.
E-mail: pierre.casellas@sanofi.com

Abbreviations: CB1, cannabinoid receptor 1; NHE-1, Na^+/H^+ exchanger NHE-1 isoform; MAPK, mitogen-activated protein kinase; CHO, Chinese hamster ovary cells; RTK, receptor tyrosine kinase; GPCR, G-protein-coupled receptor

PII: S0014-5793(99)00395-6

Na^+/H^+ exchanger activity in CHO cells transfected with CB1.

2. Materials and methods

2.1. Reagents

[γ-^{32}P]ATP (3000 Ci/mmol) was from Amersham (Les Ulis, France). PTX was purchased from Sigma Chemicals (Saint-Quentin-Fallavier, France). Insulin was purchased from Preprotech (TEBU). SR 141716A (*N*-(piperidino-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-pyrazole-3-carboxamide) and CP-55940 were synthesized in the Chemistry Department of Sanofi (Montpellier, France). G418 was from Gibco BRL (Cergy Pontoise, France). [7-^{14}C]benzoic acid was purchased from DuPont NEN. HOE694 was kindly provided by Hoechst Laboratories. The specific MEK1 inhibitor PD98059 was from Biolabs.

2.2. Expression of human CB1 receptor in CHO cells

CB1 cDNA from IM-9 [33] was amplified with a sense primer bearing a *Hin*dIII site and a Kozak consensus sequence (5′-CCACACAAGCTTGCCACCAT-GGAGGAATGCTGGGTG) and an antisense primer bearing an *Eco*RI site (5′-CCACTCGGATCCTCAGCAATCAGAGAGGTCTAG). The amplicon was digested with *Hin*dIII/*Eco*RI and inserted into p658, an expression plasmid derived from p7055 [34] in which the interleukin-2 coding sequence was replaced by a polylinker. The CB1 expression vector was transfected into CHO dihydrofolate reductase-negative cells by a modified $CaPO_4$ precipitation method [35]. CHO cells transfected with CB1 cDNA and expressing CB1 are referred to as CHO-CB1 cells. The CHO-CB1 cells were grown as monolayers in minimal essential medium supplemented with 5% dialyzed fetal calf serum, 2 mM glutamine, 40 μg/ml L-proline, 60 μg/ml Tylocine, 1 mM sodium pyruvate and 5 μg/ml gentamicin.

2.3. MAPK assay

MAPK activity was measured as described previously [15]. Briefly, cells grown to 80% confluence in 24-well plates were placed in medium containing 0.5% FCS (0% FCS when Δ^9-THC was used) for 24 h before assay. After treatment, cells were washed twice in buffer A (50 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM $Na_4P_2O_7$, 50 mM NaF, 1 mM EDTA, 20 mM glycerophosphate, 1 mM EGTA, 2 mM Na_4VO_4) and lysed for 15 min in 100 μl of buffer A containing 1% (v/v) Triton X-100, 100 U/ml aprotinin, 20 μM leupeptin, 0.2 mg/ml PMSF and 2 mM dithiothreitol. Solubilized cell extracts were centrifuged at $14\,000\times g$ for 15 min and 18 μl of supernatants (20 μg proteins) were analyzed for MAPK activity. The protein contents in the supernatants were determined using the micro BCA protein assay kit (Pierce). Phosphorylation of MAPK-specific peptide substrate was carried out at 30°C for 30 min (linear assay conditions) with [γ-^{32}P]ATP using the Biotrack p42/p44 MAP kinase enzyme system (Amersham).

2.4. Measurement of intracellular pH changes

Intracellular pH changes were estimated from the distribution of [7-^{14}C]benzoic acid, as described previously [36]. CHO-CB1 cells were grown to confluence in 24-well plates and incubated for 5 h in the serum-free medium to maintain the Na^+/H^+ exchanger in a resting state. Cells were further incubated for 1 h at 37°C in a serum- and HCO_3^--free medium containing 120 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM KCl, 5 mM glucose and 20 mM HEPES at pH 7.0. Cells were then placed for 15 min in the same medium containing 7 Bq/ml [^{14}C]benzoic acid (0.3 μCi/ml) in the presence or not of CP-55940, as described in the figure legends. Cells were then rapidly washed four times with ice-cold PBS and ^{14}C radioactivity was counted.

2.5. Transient expression and H^+-killing selection

H^+-killing selection was used for transient expression. CHO-CB1 cells were grown for 2 days (4×10^5 cells/well) in 6-well plates and transfected with 1 μg/well of mock or NHE-3 expression vector [37] by the lipofectamine technique [38]. Forty-eight hours after transfection, cells were subjected to acid-load selection [39] in the presence of 1 μM HOE694, (a selective NHE-1 blocker) to inhibit endogenous NHE1 antiporter activity, so that only transfected cells expressing the NHE3 isoform survived [40]. The surviving cells were counted 3 days later by trypan blue staining.

3. Results

3.1. Effect of CP-55940 on cytosolic pH

The effect of CP-55940 on cytosolic pH was studied in CHO cells stably transfected with CB1 (CHO-CB1) [15]. Changes in pH_i were estimated from the distribution of [^{14}C]benzoic acid as described in Section 2. CP-55940 treatment of CHO-CB1 cells induced a rapid and persistent cytoplasmic alkalinization, which peaked ($\Delta pH_i = 0.2 \pm 0.02$) at 10 min (Fig. 1A). The CP-55940-induced pH_i rise was dose-dependent, with an EC_{50} of 9 nM, which is consistent with the binding affinity of CP-55940 (Fig. 1B).

3.2. Identification of the NHE isoform affected following CP-55940 treatment

The process responsible for CP-55940-induced alkalinization was then investigated. As the experiments in Fig. 1 were performed in the absence of HCO_3^-, the observed pH_i

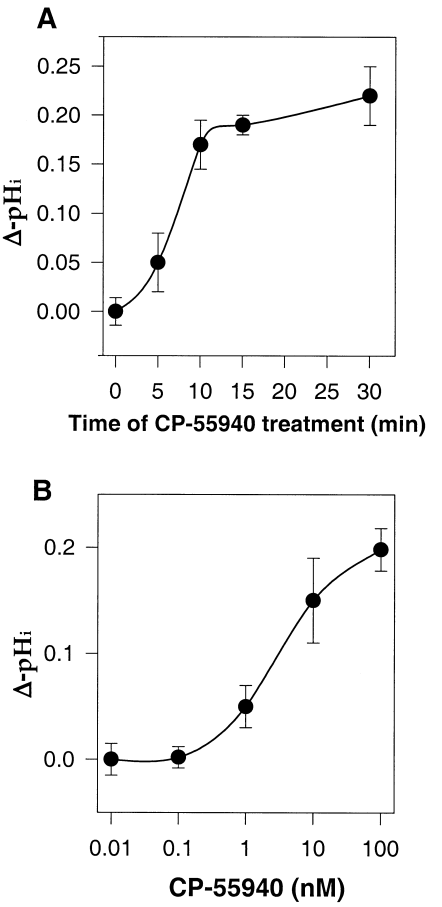

Fig. 1. Effect of CP-55940 on cytosolic pH. CHO-CB1 cells were depleted of serum for 5 h and then equilibrated for 1 h in bicarbonate-free and HEPES-buffered saline (pH 7) as described previously [31]. Then the cells were incubated for 15 min in the same medium containing 0.3 μCi/ml [^{14}C]benzoic acid in the absence or presence of CP-55940. Variations in pH_i were measured as described in [36]. ΔpH_i represents the pH_i variation relative to the control (unstimulated cells). Error bars (±S.E.M.) are based on quadruplet determinations. A: Kinetics of CP-55940 (20 nM)-induced pH_i increases. B: 15 min treatment with increasing CP-55940 concentrations.

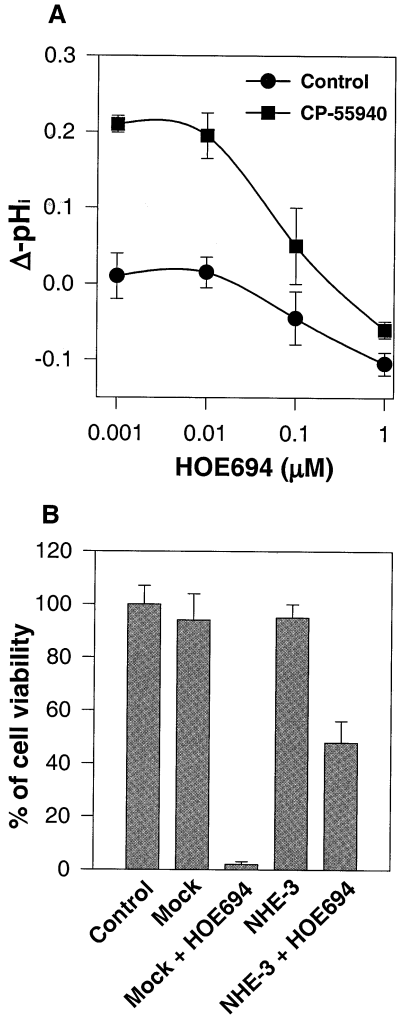

Fig. 2. CP-55940-activated pH_i increase was NHE-1 mediated. A: HOE694-induced inhibition of CP-55940-activated pH_i increase: CHO-CB1 cells were pretreated for 10 min with increasing concentrations of HOE694 (0.001–1 μM) before activation with CP-55940 (20 nM) for another 15 min. The pH_i modulation was determined as described in the legend to Fig. 1. B: CHO-CB1 cell death by H^+ suicide in the presence of HOE694 was prevented by NHE-3 transfection: CHO-CB1 cells were transiently transfected by the NHE-3 or mock expression vector as described in Section 2. Two days after transfection, cells were subjected to H^+ suicide methods in the presence or absence of HOE694 (1 μM), as described in Section 2, and cell viability was determined as % of cell growth 3 days later. The viability of NHE-3+HOE694 was underestimated since transfection efficacy was approximately 30–40%.

changes could probably not be explained by the involvement of HCO_3^-/Cl^- exchange. We therefore turned our attention to the Na^+/H^+ exchanger. NHE-1 is ubiquitously expressed and appears to be the predominant species in non-epithelial cells [25]. We thus tested whether the inhibition of NHE-1 by the NHE-1 selective inhibitor HOE694 (K_i (μM) NHE-1, 0.16; NHE-2, 5; NHE-3, 650) [40] could affect CP-55940-induced cytosol alkalinization. In Fig. 2A, we clearly show that HOE694 dose-dependently inhibited the CP-55940-induced pH_i rise, suggesting that NHE-1 is the predominant isoform mediating CP-55940-induced cytosol alkalinization. Interestingly, we observed that HOE694 also affected the basal pH_i (30% pH_i reduction in comparison to non-HOE694-treated cells), suggesting that the NHE-1 in CHO-CB1 cells had a certain degree of constitutive activity.

To confirm that CP-55940-induced cytosol alkalinization was due to NHE-1 activation, we performed acid-load selection of mock or NHE-3 transiently transfected CHO-CB1 cells in the presence or absence of HOE694. The results in Fig. 2B clearly show that the mock transfected cells survived (100%) in the absence of HOE694, whereas NHE-1 blockage by HOE694 induced 95% cell death. By contrast, NHE-3-expressing cells resisted acid-load selection even in the presence of HOE694. These results confirmed that NHE-1 is the sole Na^+/H^+ exchanger isoform functionally active in CHO-CB1.

3.3. Effect of SR 141716A on cytosolic pH

To test the specificity of the central cannabinoid receptor involvement in CP-55940-induced cytosol alkalinization, we treated the CHO-CB1 cells with the CB1 selective inverse agonist SR 141716A. The results reported in Fig. 3 clearly show that SR 141716A (100 nM) not only antagonized the ability of CP-55940 (10 nM) to induce intracellular alkalinization but also affected the basal activity of NHE-1, suggesting that the constitutive NHE-1 activation observed previously was induced by the autoactivated CB1 receptor. Furthermore, we noted that insulin stimulated the pH_i rise ($\Delta pH_i = 0.3 \pm 0.04$) and that SR 141716A also attenuated the insulin-induced NHE-1 activation (Fig. 3). These results are in accordance with those of our previous study, where SR 141716A negatively modulated G_i-dependent receptor tyrosine kinase (RTK) signaling or GPCR signaling in CHO-CB1 cells [24].

3.4. Mechanism of CP-55940-induced NHE-1 activation

To further investigate the effector pathway responsible for NHE-1 activation in CHO-CB1 cells, we first examined the effect of PTX on the NHE1 stimulation potential of CP-55940. As shown in Fig. 4, the stimulation of NHE-1 activity by CP-55940 was inhibited following PTX treatment (100 ng/ml), which was consistent with the selective coupling of CB1 to G_i/G_o proteins. We also observed that PTX treatment re-

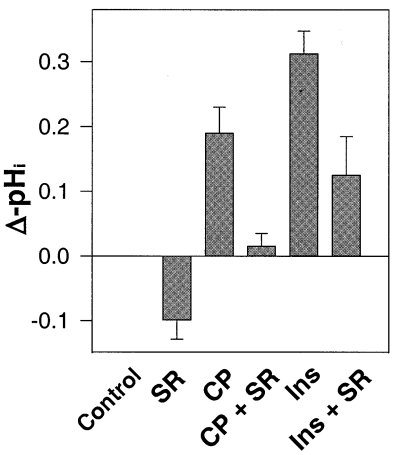

Fig. 3. Effect of SR 141716A on the cytosolic pH. Quiescent CHO-CB1 cells were pretreated with SR 141716A (100 nM) for 10 min before activation by CP-55940 (10 nM) or insulin (1 μg/ml) for 15 min. The pH_i was measured as described in the legend to Fig. 1. Data are means of quadruple determinations and are representative of experiments repeated three times.

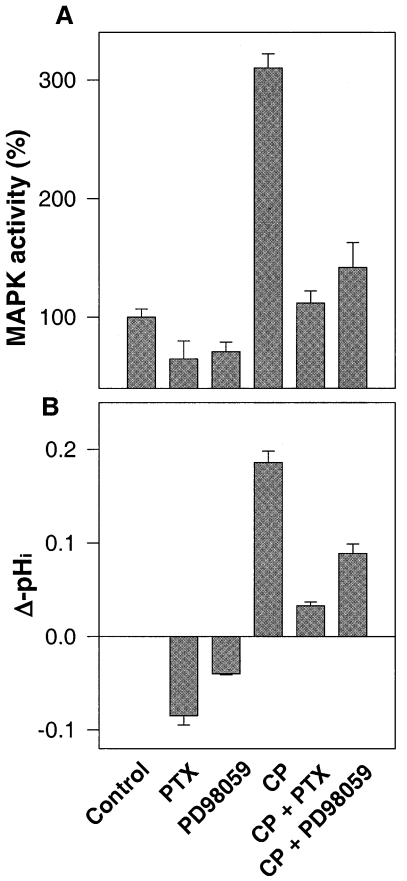

Fig. 4. Effect of PTX and PD98059 on CP-55940-induced MAPK and NHE-1 activation. Growth-arrested CHO-CB1 cells were pretreated with PTX (100 ng/ml) for 5 h or with PD98059 (10 μM) for 1 h and then activated with CP-55940 (20 nM). A: After 10 min the cells were lysed and MAPK activity was determined as described in Section 2. B: pH_i was determined 15 min later, as described in the legend to Fig. 1.

duced the basal activity of NHE-1, which is in agreement with results obtained with SR 141716A and consistent with the features of autoactivated receptors.

MAPK signaling is among the signaling events located downstream from the G protein and leading to NHE-1 activation. MAPK activation was previously shown to be linked to NHE-1 activation [31,32]. To test whether CP-55940-activated p42/p44 MAPK could regulate NHE-1 activation in CHO-CB1 cells, we used a MEK1 inhibitor PD98059 [41]. As shown in Fig. 4, PD98059 at 10 μM inhibited 80% of the activation of MAPK by CP-55940. PD98059 was also found to significantly inhibit the response of NHE-1 to both CP-55940 (50%) and insulin (45%) (not shown), highlighting the role of p42/p44 MAPK induction in CP-55940 activation of NHE-1.

4. Discussion

In this study, we clearly demonstrate that CP-55940 induced NHE-1 activation in a dose- and time-dependent manner in CHO-CB1 cells and that this induction was specifically CB1-mediated. Consequently, NHE-1 activation leads to cytosol alkalinization. We also showed that treatment of CHO-CB1 cells with the specific NHE-1 inhibitor HOE694, with the CB1 selective inverse agonist SR 141716A, or with PTX lowered the basal pH_i by more than $\Delta pH_i = 0.12 \pm 0.03$. Overall, these results indicated that autoactivated CB1 constitutively affected the basal activity of NHE-1 in CHO-CB1 cells. Furthermore, the magnitude of the CP-55940-induced pH_i rise ($\Delta pH_i = 0.2$) was underestimated (since we obtained ΔpH_i values of 0.3 when cumulating SR 141716A and CP-55940 effects). This is the first demonstration that a NHE1 exchanger can be constitutively affected by the autoactivated GPCR.

We also observed that SR 141716A could negatively modulate insulin-induced NHE-1 activation. Insulin induced NHE-1 activation via a PTX-dependent G-protein (not shown); this is consistent with our previous report, which showed that the inverse agonist SR 141716A could interfere with G_i-dependent signaling mediated by both GPCR and RTK [24].

Our observations could be extended to the CB2 receptor, since we also observed that the peripheral cannabinoid receptor CB2 stably transfected in CHO cells induced NHE-1 activation ($\Delta pH_i = 0.26 \pm 0.018$) in a PTX-sensitive manner (not shown).

GPCR modulation of NHE-1 activity is well documented. Most GPCRs stimulate NHE1 activity through coupling to G_q, G_{13} or $G_{i/o}$ GTP binding proteins [42]. On the other hand, dopamine receptors [43], somatostatin receptors [44] or constitutively active $G_{\alpha 12}$ inhibit NHE-1 activity [45].

In CHO-CB1 cells, PTX prevented CP-55940-induced MAPK and NHE-1 activation, indicating $G_{i/o}$ protein involvement.

The MEK1-specific inhibitor PD98059 (at 10 μM) prevented CP-55940-induced MAPK activation by up to 80%, but inhibited CP-55940-induced NHE-1 activation by only 50%. The residual MAPK activity could be sufficient to produce NHE-1 activation. Alternatively these results could also suggest that, in addition to the $G_{i/o}$ p42/p44 MAPK signaling cascade, CB1 induced NHE-1 activation via another $G_{i/o}$-dependent but p42/p44 MAPK-independent signaling pathway. $G_{i/o}$-dependent but p42/p44 MAPK-independent signaling activation of NHE1 has been implicated in NHE-1 activation by 5-HT_{1A} receptors transfected in CHO cells [46]. CB1 might use another signaling cascade such as cdc42-MEKK1, which has been shown to be involved in NHE-1 activation [47]. There is also possible involvement of NHERF (Na^+/H^+ exchanger regulatory factor) PDZ proteins which were shown to be involved in the regulation of β_2 adrenoreceptor-induced NHE-3 activation by direct interaction with the DSLL (D S/T X L consensus) motif in the C-terminal tail of β_2 adrenoreceptors [48,49]. Analysis of the CB1 C-terminal tail showed a DTSV motif, which is a potential binding site of PDZ proteins.

As the above results were obtained with CHO cells overexpressing CB1, we next examined whether they could be extended to human cell lines expressing endogenous CB1. We thus used the U373MG astrocytoma cell line which expresses CB1 [15,16]. We previously observed that CB1 stimulation induced MAPK activation and Krox24 expression. In U373MG cells, we observed a slight increase in pH_i ($\Delta pH_i = 0.095 \pm 0.02$) after CP-55940 treatment (data not shown).

The central nervous system is a well-known target of systemic acid-base disorders. There is growing evidence that rapid and local pH transients may alter neuronal excitability. A

wide variety of neuronal signaling mechanisms are pH-dependent, including membrane voltage- and ligand-gated ion channels, transmitter uptake through transporters, intracellular signal transduction, and intracellular communication via gap junctions [50]. Although we did not analyze the effect of cannabinoid ligands on NHE-1 activity in neuronal cells, we hypothesize that cannabinoid ligands may regulate neuronal excitability by modulating NHE-1 activity. This hypothesis could be related to the observation that the CB1 agonist Δ^9-THC and endocannabinoids, such as anandamide, lowered neuronal excitability by inhibiting neurotransmitters (glutamate, dopamine) release [51].

References

[1] Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C. and Bonner, T.I. (1990) J. Neurosci. 346, 561–564.
[2] Gerard, C.M., Mollereau, C., Vassart, G. and Parmentier, M. (1991) Biochem. J. 279, 129–134.
[3] Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R. and Rice, K.C. (1991) J. Neurosci. 11, 563–583.
[4] Matsuda, L.A., Bonner, T.I. and Lolait, S.J. (1993) J. Comp. Neurol. 327, 535–550.
[5] Kaminski, N.E., Abood, M.E., Kessler, F.K., Martin, B.R. and Schatz, A.R. (1992) Mol. Pharmacol. 42, 736–742.
[6] Bouaboula, M., Rinaldi, M., Carayon, P., Carillon, C., Delpech, B., Shire, D., Le Fur, G. and Casellas, P. (1993) Eur. J. Biochem. 214, 173–180.
[7] Munro, S., Thomas, K.L. and Abu-Shaar, M. (1993) J. Neurosci. 365, 61–65.
[8] Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G. and Casellas, P. (1995) Eur. J. Biochem. 232, 54–61.
[9] Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam, R. (1992) Science 258, 1946–1949.
[10] Sugiura, T., Kodaka, T., Kondo, S., Tonegawa, T., Nakane, S., Kishimoto, S., Yamashita, A. and Waku, K. (1996) Biochem. Biophys. Res. Commun. 229, 58–64.
[11] Stella, N., Schweitzer, P. and Piomelli, D. (1997) J. Neurosci. 388, 773–778.
[12] Howlett, A.C. and Fleming, R.M. (1984) Mol. Pharmacol. 26, 532–538.
[13] Howlett, A.C. (1985) Mol. Pharmacol. 27, 429–436.
[14] Felder, C.C., Joyce, K.E., Briley, E.M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A.L. and Mitchell, R.L. (1995) Mol. Pharmacol. 48, 443–450.
[15] Bouaboula, M., Poinot-Chazel, C., Bourrie, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., Le Fur, G. and Casellas, P. (1995) Biochem. J. 312, 637–641.
[16] Bouaboula, M., Poinot-Chazel, C., Marchand, J., Canat, X., Bourrie, B., Rinaldi-Carmona, M., Calandra, B., Le Fur, G. and Casellas, P. (1996) Eur. J. Biochem. 237, 704–711.
[17] Bouaboula, M., Bourrie, B., Rinaldi-Carmona, M., Shire, D., Le Fur, G. and Casellas, P. (1995) J. Biol. Chem. 270, 13973–13980.
[18] Mailleux, P., Verslype, M., Preud'homme, X. and Vanderhaeghen, J.J. (1994) NeuroReport 5, 1265–1268.
[19] Glass, M. and Dragunow, M. (1995) NeuroReport 6, 241–244.
[20] Mackie, K. and Hille, B. (1992) Proc. Natl. Acad. Sci. USA 89, 3825–3829.
[21] Mackie, K., Lai, Y., Westenbroek, R. and Mitchell, R. (1995) J. Neurosci. 15, 6552–6561.
[22] Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G. and Caput, D. (1994) FEBS Lett. 350, 240–244.
[23] Rinaldi-Carmona, M., Pialot, F., Congy, C., Redon, E., Barth, F., Bachy, A., Breliere, J.C., Soubrie, P. and Le Fur, G. (1996) Life Sci. 58, 1239–1247.
[24] Bouaboula, M., Perrachon, S., Milligan, L., Canat, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Pecceu, F., Lupker, J., Maffrand, J.P., Le Fur, G. and Casellas, P. (1997) J. Biol. Chem. 272, 22330–22339.
[25] Orlowski, J. and Grinstein, S. (1997) J. Biol. Chem. 272, 22373–22376.
[26] Sardet, C., Franchi, A. and Pouyssegur, J. (1989) Cell 56, 271–280.
[27] Noel, J. and Pouyssegur, J. (1995) Am. J. Physiol. 268, C283–C296.
[28] Aronson, P.S., Nee, J. and Suhm, M.A. (1982) Nature 299, 723–726.
[29] Moolenaar, W.H., Tsien, R.Y., van der Saag, P.T. and de Laat, S.W. (1983) J. Neurosci. 304, 645–648.
[30] Paris, S. and Pouyssegur, J. (1984) J. Biol. Chem. 259, 10989–10994.
[31] Bianchini, L., L'Allemain, G. and Pouyssegur, J. (1997) J. Biol. Chem. 272, 271–279.
[32] Aharonovitz, O. and Granot, Y. (1996) J. Biol. Chem. 271, 16494–16499.
[33] Shire, D., Carillon, C., Kaghad, M., Calandra, B., Rinaldi-Carmona, M., Le Fur, G., Caput, D. and Ferrara, P. (1995) J. Biol. Chem. 270, 3726–3731.
[34] Miloux, B. and Lupker, J.H. (1994) Gene 149, 341–344.
[35] Graham, F.L. and Eb, A.J. (1973) Virology 54, 536–539.
[36] L'Allemain, G., Paris, S. and Pouyssegur, J. (1984) J. Biol. Chem. 259, 5809–5815.
[37] Noel, J., Roux, D. and Pouyssegur, J. (1996) J. Cell Sci. 109, 929–939.
[38] Hawley-Nelson, P., Ciccarone, V., Gebeyehu, G., Jesse, J. and Felgner, P.L. (1993) FOCUS 15, 73.
[39] Pouyssegur, J., Sardet, C., Franchi, A., L'Allemain, G. and Paris, S. (1984) Proc. Natl. Acad. Sci. USA 81, 4833–4837.
[40] Counillon, L., Scholz, W., Lang, H.J. and Pouyssegur, J. (1993) Mol. Pharmacol. 44, 1041–1045.
[41] Dudley, D.T., Pang, L., Decker, S.J., Bridges, A.J. and Saltiel, A.R. (1995) Proc. Natl. Acad. Sci. USA 92, 7686–7689.
[42] Garnovskaya, M.N., Gettys, T.W., van Biesen, T., Prpic, V., Chuprun, J.K. and Raymond, J.R. (1997) J. Biol. Chem. 272, 7770–7776.
[43] Ganz, M.B., Pachter, J.A. and Barber, D.L. (1990) J. Biol. Chem. 265, 8989–8992.
[44] Hou, C., Gilbert, R.L. and Barber, D.L. (1994) J. Biol. Chem. 269, 10357–10362.
[45] Lin, X., Voyno-Yasenetskaya, T.A., Hooley, R., Lin, C.Y., Orlowski, J. and Barber, D.L. (1996) J. Biol. Chem. 271, 22604–22610.
[46] Garnovskaya, M.N., Mukhin, Y. and Raymond, J.R. (1998) Biochem. J. 330, 489–495.
[47] Hooley, R., Yu, C.Y., Symons, M. and Barber, D.L. (1996) J. Biol. Chem. 271, 6152–6158.
[48] Hall, R.A., Ostedgaard, L.S., Premont, R.T., Blitzer, J.T., Rahman, N., Welsh, M.J. and Lefkowitz, R.J. (1998) Proc. Natl. Acad. Sci. USA 95, 8496–8501.
[49] Hall, R.A., Premont, R.T., Chow, C.W., Blitzer, J.T., Pitcher, J.A., Claing, A., Stoffel, R.H., Barak, L.S., Shenolikar, S., Weinman, E.J., Grinstein, S. and Lefkowitz, R.J. (1998) J. Neurosci. 392, 626–630.
[50] Di Marzo, V., Melck, D., Bisogno, T. and De Petrocellis, L. (1998) Trends Neurosci. 21, 521–528.
[51] Takahashi, K.I. and Copenhagen, D.R. (1996) Neurosci. Res. 24, 109–116.