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# Molecular study of the $Na^+/Ca^{2+}$ exchanger in bovine adrenal chromaffin cells

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To identify the $Na^+/Ca^{2+}$ exchanger expressed in bovine chromaffin cells, the *ncx* gene was cloned from a bovine chromaffin cell cDNA library. Five partial clones were obtained and their nucleotide sequences showed that there were at least three isoforms containing different intracellular loops. The 3′-untranslated region was the same in all the clones. To examine the $Na^+/Ca^{2+}$ exchange activity of the clones, full-length *ncx1* genes were constructed by replacing the corresponding region of bovine cardiac *ncx1* clone p17 with the different regions from two bovine chromaffin cell clones; these were designated p17c and p17h. p17h, but not p17c, showed $Na^+/Ca^{2+}$ exchange activity when expressed in Chinese hamster ovary cells and *Xenopus* oocytes. The expressed exchange activity of p17 was inhibited by 8-bromoadenosine 3′:5′-cyclic monophosphate (8-Br-cAMP) but was not affected by PMA. However, the activity of p17h was inhibited by PMA but enhanced by 8-Br-cAMP. The agents that changed the activity of protein kinase C and cAMP-dependent protein kinase modulated the endogenous $Na^+/Ca^{2+}$ exchange current of chromaffin cells in a manner similar to that of p17h. Our results suggest that the p17h clone is the major isoform of the exchanger in chromaffin cells and is similar to the major *ncx1* isoform in kidney. The exchange activity could be regulated by phosphorylation, and the variable region in the intracellular loop is important for the different effects of phosphorylation on the different isoforms.

## INTRODUCTION

Among the mechanisms known for the maintenance of intracellular $Ca^{2+}$ homeostasis, the $Na^+/Ca^{2+}$ exchanger (NCX) in the plasma membrane is one of the major mechanisms in bringing the cytosolic free $Ca^{2+}$ concentration to the resting level in bovine adrenal chromaffin cells [1–4]. NCX is an electrogenic transporter with a $Na^+/Ca^{2+}$ stoichiometry of 3:1. NCX can function in either forward mode (extracellular $Na^+$ in exchange for intracellular $Ca^{2+}$) or reverse mode (intracellular $Na^+$ in exchange for extracellular $Ca^{2+}$) depending on the membrane potential and the gradients of $Na^+$ and $Ca^{2+}$ across the plasma membrane [5,6].

At least three genes for NCX have been cloned [7–9]: *ncx1* is expressed predominantly in heart and is also abundant in many other tissues; *ncx2* and *ncx3* are highly restricted to the brain and skeletal muscles. The exchanger encoded by *ncx1* has been studied extensively. *ncx1* was first cloned from dog cardiac tissue, which encodes a protein of 970 residues and comprises a cleaved signal sequence followed by a short glycosylated extracellular region, a domain of five hydrophobic transmembrane segments, a long cytoplasmic loop, and a final region of six transmembrane segments (see Figure 1) [7,10]. This predicted conformation is applicable to all NCX forms cloned from different species. The only structural diversity among different NCX1 isoforms is in a small variable region towards the C-terminal end of the cytoplasmic loop, apparently as a consequence of alternative splicing of six exons including two mutually exclusive exons, A and B, and four cassette exons, C, D, E and F (see Figure 1) [11,12]. In addition, the 5′-untranslated region seems to possess another splicing site that is proposed to be responsible for the regulation of the expression of *ncx* in a tissue-specific fashion [11,13].

The activity of cardiac NCX is known to be positively regulated by cytoplasmic $Ca^{2+}$ and $MgATP^{2-}$ and inactivated by cytoplasmic $Na^+$. All regulation can be abolished by treating the cytoplasmic side of the excised membrane patch with chymotrypsin [14]. Moreover, the exchange activity remains in mutants from which most of the cytoplasmic loop has been removed [15]. It is therefore thought that the large cytoplasmic loop of NCX is responsible for the regulation of NCX activity. Through mutagenesis studies, several regions in the cytoplasmic loop have been identified as being responsible for the regulation of exchange activity by $Na^+$ and $Ca^{2+}$ [16–18]. However, the effect of the small variable region, which accounts for the structural differences in various NCX1 isoforms, on the properties of the exchanger is not clear.

The role of phosphorylation in the regulation of the NCX activity remains unclear. For example, it has been reported that the stimulation of cardiac NCX activity by $MgATP^{2-}$ does not involve phosphorylation in giant excised patches of sarcolemma [19]. However, in CCL39 cells overexpressing canine cardiac NCX1 protein and in rat neonatal cardiomyocytes, the up-regulation of NCX1 activity by protein kinase C (PKC) is correlated with the phosphorylation of NCX1 [20]. The increase in the phosphorylation level of the cell does not always increase NCX activity. In bovine chromaffin cells the activity of NCX is inhibited by agents that promote protein phosphorylation [3].

Although the physiological importance of NCX in bovine adrenal chromaffin cells has been recognized, the molecular identity of NCX in these cells has not been characterized. In the present study we isolated and characterized the *ncx* clones from a bovine chromaffin cell cDNA library. Our results show that the isoform in bovine chromaffin cells was regulated differently by different protein kinases and that the form of regulation was different from that of the bovine cardiac NCX1 protein.

Abbreviations used: 8-Br-cAMP, 8-bromoadenosine 3′:5′-cyclic monophosphate; CHO, Chinese hamster ovary; NCX, $Na^+/Ca^{2+}$ exchanger; PKA, cAMP-dependent protein kinase; PKC, protein kinase C.

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## MATERIALS AND METHODS

### Cloning of the NCX from bovine chromaffin cells

A bovine adrenal chromaffin cell cDNA library in λgt11 (Clontech, Palo Alto, CA, U.S.A.) was screened with a PCR product coding for the conserved region in the large cytoplasmic loop of *ncx1* (see Figure 1). The PCR product of approx. 800 bp was obtained with primers NC1 (5′-CTTAGATGGTGCTCTGGTTCTGGAGG-3′, nt 1167–1192) and NC2 (5′-GGAACGATAACATTTCCTCGAGCTCC-3′, nt 1951–1976) by using first-strand cDNA synthesized from mRNA isolated from bovine chromaffin cells as template; the conditions were: 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 3 min (30 cycles). Two clones, c and e, were obtained from the first screening. A second screening was performed with clone c as a probe. Three more clones, d, h and m, were obtained. These clones were then subcloned into the *Eco*RI sites of the pTZ19R vector for sequencing; clones d and m seemed to be identical. The sizes and corresponding regions of these clones to bovine cardiac *ncx1* clone p17 are shown in Figure 1.

### PCR amplification of *ncx* coding region

Oligonucleotides NC3 (5′-AGGTTGGGACAGTTGGAACTCTGCCA-3′, nt 229–254) and NC4 (5′-GCCTTCTCTCTCCGGCCCTAGTACAG-3′, nt 3308–3343) were used as PCR primers to synthesize the entire coding region of *ncx1* cDNA by using the double-stranded cDNA obtained from the Marathon Kit as template. The thermal regime was 94 °C for 5 min (1 cycle), 94 °C for 30 s, and 68 °C for 4 min (25 cycles). A band of approx. 3 kb was obtained and subcloned into the pCR 2.1 vector. To amplify the variable region, primers NC5 (5′-AAGACCATTGAGGGGACCGC-3′, nt 1981–2000) and NC6 (5′-TTGCAAATGTGTCTGGCACT-3′, nt 2796–2815) were used. The thermal regime was 94 °C for 5 min (1 cycle), 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 3 min (25 cycles). A discrete band of approx. 700 bp was obtained and subcloned into the pCR2.1 vector for sequencing.

### Construction of full-length bovine chromaffin cell NCX protein

To obtain the full-length bovine chromaffin NCX protein, the corresponding region of the bovine cardiac *ncx1* clone p17 was replaced by the regions between the restriction sites of *Sac*I and *Sca*I of clone c and *Sac*I and *Sac*II of clone h and the full-length clones obtained were designated p17c and p17h respectively (see Figure 1). The amino acid sequence of the proteins expressed from p17h would be the same as that of native bovine chromaffin NCX. The constructs and p17 were then subcloned into the pcDNAIII vector (InVitrogen, Leek, The Netherlands) for transfection and transcription *in vitro*.

### Expression of $Na^+/Ca^{2+}$ exchange activity in Chinese hamster ovary (CHO) cells

CHO cells were plated at a density of $5 \times 10^5$ cells per 100 mm dish. Before transfection the cells were incubated overnight with McCoy's 5A medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum. The transfection was performed with Lipofectamine (Gibco BRL, Gaithersburg, MD, U.S.A.) in accordance with the manufacturer's instructions. Cells were first rinsed with serum-free McCoy's 5A medium and then incubated in serum-free medium supplemented with premixed 25 μl of Lipofectamine and 12 μg of column-purified (Qiagen, Santa Clarita, CA, U.S.A.) plasmid DNA. The transfection mixture was removed after 5 h at 37 °C and the cells were incubated with medium containing 10% (v/v) serum for 48 h before the isolation of membranes.

The membrane fractions were collected by homogenizing the cells in 0.25 M SME buffer [0.25 M sucrose/10 mM Mops/5 mM EDTA (pH 7.0)] containing 200 μM PMSF. Unbroken cells and large organelles were removed by low-speed centrifugation (1000 ***g*** for 10 min). The supernatant was then diluted with 2 vol. of $Na^+$ buffer [150 mM NaCl/2 mM $MgCl_2$/10 mM Hepes (pH 7.4)] and centrifuged at 100000 ***g*** for 2 h. The pellet was resuspended in $Na^+$ buffer and used for the assay of $Na^+/Ca^{2+}$ exchange activity by $^{45}Ca^{2+}$ uptake in a $K^+$-rich or $Na^+$-rich solution as previously described [21].

### Expression of $Na^+/Ca^{2+}$ exchange activity in *Xenopus* oocytes

Clones p17, p17c and p17h were transcribed *in vitro* with T7 RNA polymerase (T7 mMessage mMachine Kit; Ambion, Austin, TX, U.S.A.). Transcribed RNA (50 nl; 1 ng/nl) was injected into *Xenopus* oocytes and incubated at 18 °C in modified Barth's solution [88 mM NaCl/1 mM KCl/2.4 mM $NaHCO_3$/0.82 mM $MgSO_4$/0.33 mM $Ca(NO_3)_2$/0.41 mM $CaCl_2$/5 mM Hepes (pH 7.1)] for 3 days. The oocytes were loaded with $Na^+$ in nominally $Ca^{2+}$-free modified Barth's solution containing 30 μM nystatin at 4 °C for 30 min and assayed for $Na^+/Ca^{2+}$ exchange activity by $^{45}Ca^{2+}$ uptake in solution containing either 90 mM KCl or 90 mM NaCl, as previously described [22]. The $Na^+/Ca^{2+}$ exchange activity was taken as the difference between the $^{45}Ca^{2+}$ taken up in 90 mM KCl and 90 mM NaCl.

### Immunofluorescence staining

Cells plated on coverslips were fixed in solution containing methanol/acetone (1:1, v/v) for 2 min. After being blocked with 5% (v/v) skimmed milk for 30 min in PBS [137 mM NaCl/19.3 mM KCl/8 mM $Na_2HPO_4$/1.8 mM $KH_2PO_4$ (pH 7.4)], the cells were incubated overnight at 4 °C with anti-cardiac NCX1 monoclonal rat IgM antibody (Affinity Bioreagents, Golden, CO, U.S.A.) at 1:400 dilution. Cells were then rinsed with PBS (three times in 30 min) and incubated with the secondary antibody, fluorescein isothiocyanate-conjugated goat IgG against mouse IgM μ-unit (Ancell Corp., Bayport, MN, U.S.A.), at 1:500 dilution for 1 h, followed by three washes with PBS. In the control experiments, cells were incubated with the secondary antibody only. Cells were then examined with a fluorescence microscope (Axiophot; Zeiss).

### Electrophysiological measurement of the NCX current

The inward current of the exchanger was measured by using whole-cell voltage clamp as described by Kimura et al. [23]. Cells were incubated in a bath solution containing 140 mM *N*-methylglucamine, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM $BaCl_2$, 2 mM $CsCl_2$, 10 μM nifedipine, 10 μM verapamil, 2 μM tetrodotoxin and 5 mM Hepes, pH 7.4. The patch pipette solution contained 166 mM CsOH, 42 mM aspartic acid, 20 mM tetraethylammonium chloride, 10 mM $MgATP^{2-}$, 5 mM creatine phosphate, 3 mM $MgCl_2$, 2 mM $CaCl_2$, 1.58 mM EGTA and 5 mM Hepes, pH 7.4. The membrane potential was held at −70 mV. Inward current was induced by puffing a $Na^+$-rich solution containing 140 mM NaCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$ and 5 mM Hepes, pH 7.4, on to the cell.

## RESULTS

### Primary structure of the bovine chromaffin NCX

Among the five clones obtained from the bovine chromaffin cell cDNA library, there were three different sequences at the C-terminus of the intracellular loop that can be explained by alternative splicing of the different exons such as that shown in rabbit and rat *ncx1* [11,12]. Figure 1 shows the sizes and corresponding positions of these five clones, e, c, d, m and h, relative to the bovine cardiac *ncx1* clone p17 [24]. In the C-terminal variable region of the intracellular loop, four clones (c, d, m and h) contained exons B and D, whereas clone e contained only exon B. The nucleotide sequence of exon B is shown in Figure 2. The nucleotide sequences, including the 3′-untranslated region, of these clones were identical with that of the bovine cardiac *ncx1* (results not shown). It seems that clone h is similar to an isoform of *ncx1*, NACA3, obtained from kidney [25] (Figure 2). In contrast, the cardiac clone p17 contained exon A and cassette exons C, D, E and F. It is important to note that clone c might represent a novel isoform that has an additional in-frame deletion of 39 residues covering a highly negatively charged C-terminal end of the intracellular loop and the putative sixth transmembrane domain (Figure 2).

To identify the isoforms in bovine chromaffin cells, specific primers to cover the variable C-terminus of the intracellular loop region or the entire coding regions were used to amplify the cDNA synthesized from the purified bovine chromaffin cell mRNA. Both PCR products showed a single species with the sequence of the variable region identical with that of clone h; the rest of the sequences were identical with that of the cardiac clone p17 (results not shown). It is likely that clone h represents the major isoform present in bovine chromaffin cells. The two other isoforms, clones c and e, might have constituted only a small fraction of *ncx1* transcripts and could not be amplified by the PCR reaction, or the amount was too small to be subcloned.

### Transient expression of $Na^+/Ca^{2+}$ exchange activity in CHO cells

To study the function of NCX1 from chromaffin cells, the exchanger clones were transiently expressed in CHO cells, which have very little endogenous $Na^+/Ca^{2+}$ exchange activity. CHO cells were transfected with p17, p17c and p17h; their membranes were then isolated and assayed for $Na^+/Ca^{2+}$ exchange activity. The membranes isolated from CHO cells transfected with p17 and p17h had significantly higher $Na^+/Ca^{2+}$ exchange activity than membranes from cells transfected with p17c and pcDNAIII vector only (Table 1). The results showed that clone h from the bovine chromaffin cell could be expressed as a functional NCX. Immunofluorescence staining showed that both p17 and p17h were present in the plasma membrane of the transiently transfected CHO cells, whereas p17c was present predominantly in the cytosol (Figure 3). It seems that the p17c product might not be correctly transported to the plasma membrane because of the lack of a putative sixth transmembrane segment, thus showing no activity.

### Expression of $Na^+/Ca^{2+}$ exchange activity in *Xenopus laevis* oocytes

To characterize further the activities of these *ncx1* clones, RNA transcribed *in vitro* with T7 RNA polymerase from clones p17, p17c or p17h were injected into *X. laevis* oocytes and then assayed for $Na^+/Ca^{2+}$ exchange activity. The results showed high levels of exchange activity for p17 and p17h RNA but no significant activity was detected in oocytes injected with p17c RNA or water (Table 2).

### Possible regulation of NCX activity by phosphorylation

To study the possible effect of phosphorylation on the exchanger activity expressed in oocytes, kinase activators or inhibitors were injected into oocytes before assay for exchange activity (Figure 4). PMA, an activator of PKC, decreased the exchange activity of p17h to $27 \pm 16\%$ (given as mean $\pm$ S.E.M.) of the control but had no effect on p17. In contrast, 8-bromoadenosine 3′:5′-cyclic monophosphate (8-Br-cAMP), an activator of cAMP-dependent protein kinase (PKA), increased the exchange activity of p17h to $145 \pm 5\%$ of the control but inhibited the activity of p17 to

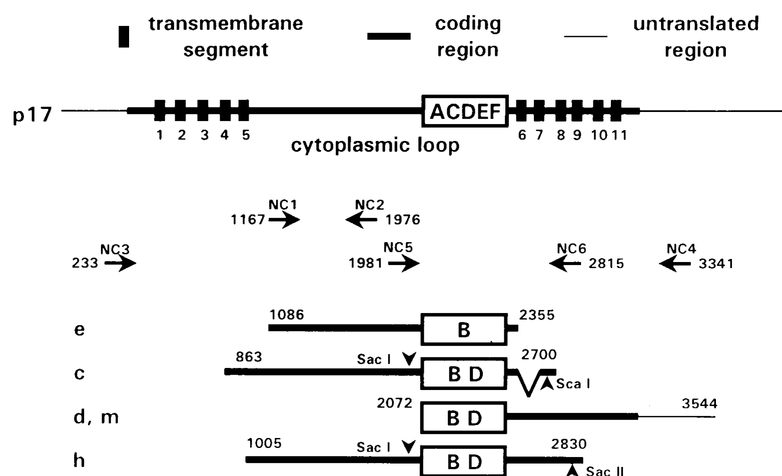


**Figure 1 Comparison of the NCX clones obtained from the cDNA library of bovine adrenal chromaffin cells and the bovine cardiac p17 clone**

The positions of the five clones (c, d, e, h and m) obtained from the chromaffin cell cDNA library relative to that of the cardiac p17 clone are shown. A, B, C, D, E and F represent the exons in the cytoplasmic loop. Clone c has a gap between nt 2526 and 2643 that covers the putative transmembrane segment 6. Two full-length exchanger clones, p17c and p17h, were constructed by replacing the corresponding region in the p17 clone with the regions between *Sac*I and *Sca*I in clone c and *Sac*I and *Sac*II in clone h. The positions and directions of the primers (NC1–6) used in this study are also shown. The numbering of the nucleotide is based on the cardiac p17 clone.

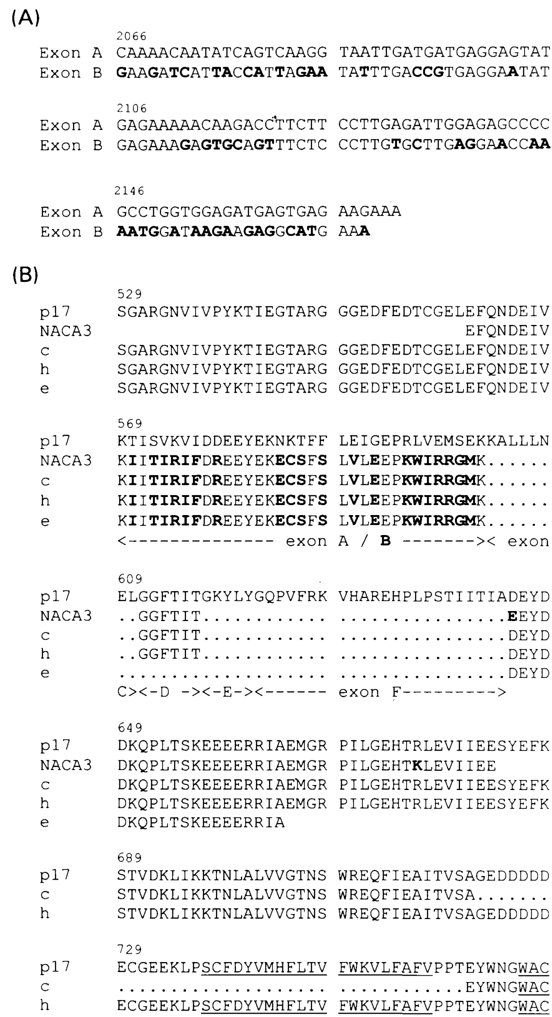


**Figure 2 Comparison of the nucleotide and deduced amino acid sequences in the variable region of the NCX clones obtained from bovine chromaffin cells and that of bovine cardiac p17 clone and rabbit kidney NACA3 isoform**

(**A**) Nucleotide sequence of exons A and B. The nucleotides that are different between the two sequences are shown in bold. (**B**) Alignment of deduced amino acid sequences. The amino acid residues that are different from the p17 clone are shown in bold. The sequence of the rabbit NACA3 isoform is from [25]. The positions of six exons (A–F) are indicated. Dots denote the gaps introduced to align the sequences. The putative transmembrane regions are underlined. The sequence of clone e is identical with that of clone h except that residues 611–616 are missing from clone e. The numbering of the residues is based on the cardiac p17 clone.

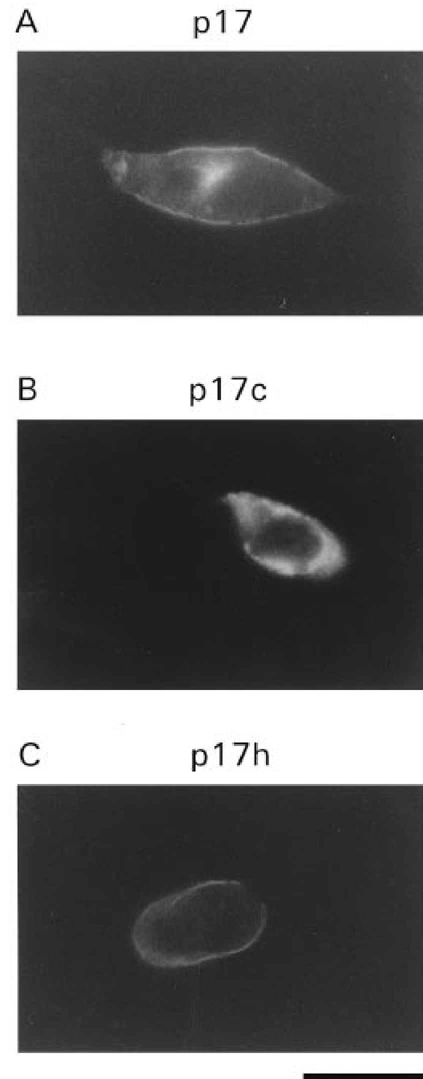


**Figure 3 Immunofluorescence images of the CHO cells transfected with p17, p17c and p17h**

CHO cells were transfected with p17, p17c and p17h; the cells were then stained with antibody against the intracellular loop of the NCX. Scale bar, 20 $\mu$m.

**Table 1 Expression of NCX in CHO cells**

CHO cells were transiently transfected with p17, p17c or p17h. The $Na^+$-gradient-dependent $^{45}Ca^{2+}$ uptake in 12 min by the crude membrane fractions was then measured. NCX activity is taken as the difference between the $^{45}Ca^{2+}$ taken up in the absence and in the presence of a $Na^+$ gradient. Results are means $\pm$ S.E.M. for six membrane preparations.

| Clone | $Na^+$-dependent $^{45}Ca^{2+}$ uptake (pmol of $Ca^{2+}$/mg of protein) |
|---|---|
| pcDNAIII | 4.3 ± 37.8 |
| p17 | 437.6 ± 82.8 |
| p17c | −9.5 ± 41.8 |
| p17h | 325.5 ± 28.3 |

**Table 2 Expression of NCX in *Xenopus* oocytes**

Clones p17, p17c and p17h were transcribed *in vitro* with T7 RNA polymerase. The RNA transcribed *in vitro* (50 ng) was injected into *Xenopus* oocytes and then incubated for 3 days before assay. NCX activity was measured as the difference between the $Na^+$-gradient-dependent and $Na^+$-gradient-independent $^{45}Ca^{2+}$ uptakes. The experiments were performed three times with different batches of oocytes. Six oocytes were used for each data point, presented as mean $\pm$ S.E.M. Results from one representative experiment are shown.

| Condition | $^{45}Ca^{2+}$ uptake (pmol of $Ca^{2+}$/10 min per oocyte) | |
|---|---|---|
| | $Na^+$-independent | $Na^+$-dependent |
| Water | 2.1 ± 0.2 | 1.5 ± 0.2 |
| p17 | 4.0 ± 0.9 | 36.5 ± 2.6 |
| p17c | 1.1 ± 0.1 | 1.6 ± 0.3 |
| p17h | 3.9 ± 0.5 | 58.6 ± 9.5 |

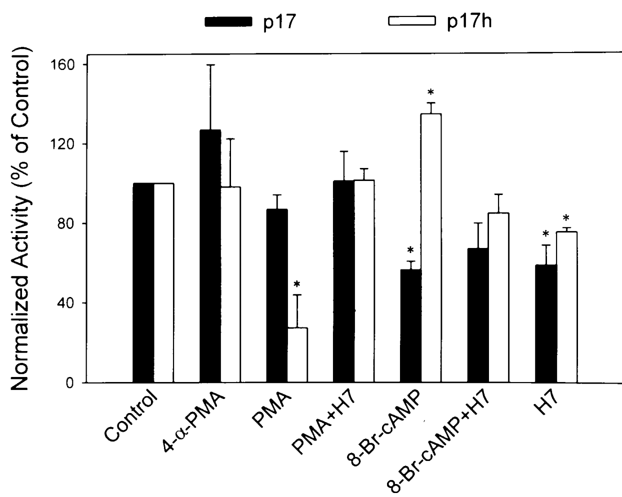


**Figure 4 Effects of phosphorylation on NCX activity**

Clones p17 and p17h were expressed in *Xenopus* oocytes and exchanger activity was measured as described in the legend to Table 2. The oocytes were injected with various agents as indicated. The approximate final concentrations of the agents used were as follows: PMA, 400 nM; 4-$\alpha$-PMA, 400 nM; 8-Br-cAMP, 0.2 mM; H7, 100 $\mu$M. The experiments were performed three times with different batches of oocytes. Six oocytes were used for each data point. Results are presented as means $\pm$ S.E.M. for three independent experiments. *Significantly different ($P < 0.05$) from control oocytes by Student's *t*-test.

$56 \pm 4$ %. To confirm that these modulation effects came directly from the activation of the specific protein kinases, 4-$\alpha$-PMA, an inactive analogue of PMA, and H7, an inhibitor of PKA and PKC, were used. The injection of 4-$\alpha$-PMA into oocytes did not have any significant effect on the modulation of $Na^+/Ca^{2+}$ exchange activity. Co-injection of H7 with kinase activator into oocytes reversed the effect of these activators, although injection of H7 alone slightly inhibited $Na^+/Ca^{2+}$ exchange activities.

To compare the properties of the cloned exchanger with those of the endogenous exchanger of the chromaffin cells, the effects of various kinase activators and inhibitors on the exchange current of the chromaffin cells were studied. Figure 5 shows that the agents that affect the activities of PKA and PKC changed the inward current of the exchanger in a similar trend to that of the expressed p17h. In 4 min after the patched membrane had been broken, 8-Br-cAMP increased the exchange current to $234 \pm 28$ %, and the PKA inhibitor KT5720 reversed the effect of 8-Br-cAMP. In contrast, perfusion of the catalytic subunit of PKC inhibited the exchange current by 20 %; calphostin C, a PKC inhibitor, enhanced the exchange current by approx. 70 %.

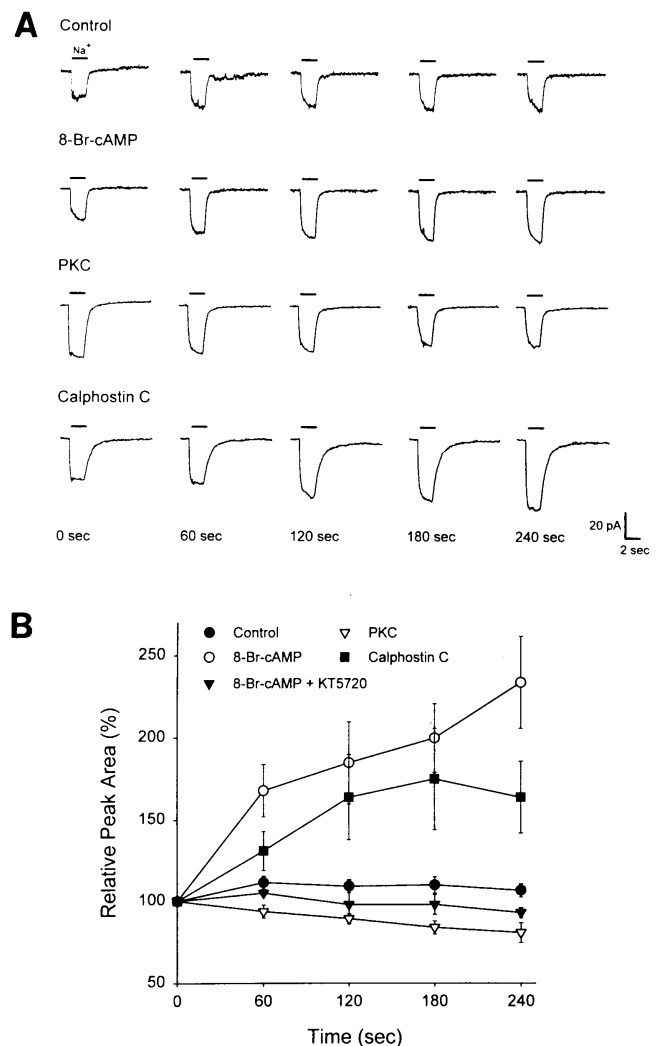


**Figure 5 NCX current in bovine adrenal chromaffin cells**

8-Br-cAMP (100 $\mu$M), the catalytic subunit of PKC (9.5 m-unit/ml; PKC), calphostin C (500 nM) or KT5720 (500 nM) was included in the pipette solution when required. The external $Na^+$-induced inward current in internal $Ca^{2+}$-loaded cells was measured immediately (0 s) and at various times as indicated after the patched membrane had been broken. (**A**) Representative traces; (**B**) averaged peak currents (means $\pm$ S.E.M.) from six to nine cells recorded as in (**A**).

## DISCUSSION

In the present study we obtained three different NCX clones from a bovine adrenal chromaffin cell cDNA library. They seem to be the gene product of *ncx1*. The major difference between the NCX isoforms from bovine cardiac and chromaffin cells resides at the C-terminus of the intracellular loop. One of the clones, h, showed $Na^+/Ca^{2+}$ exchange activity when expressed in CHO cells and *Xenopus* oocytes. The expressed exchange activity could be differently modulated by different protein kinases and the form of the regulation was similar to that of the endogenous exchanger current of the bovine chromaffin cell but different from that of the cardiac clone p17. It is likely that clone h is the major NCX present in the plasma membrane of chromaffin cells.

It is now clear that the variable region of *ncx1* clones is a result of alternative splicing; however, the influence of this alternative splicing region on the regulation of the activity of NCX is not known. In the present study we showed that the activation of PKC and PKA had different effects on the expressed cardiac p17 and chromaffin cell p17h clones. The only difference between clones p17 and p17h was in the variable region. It is possible that the phosphorylation sites reside in this region and have a direct effect on the exchange activity or that phosphorylation occurs in other regions or molecules but interacts with this variable region to exert its effect (see below).

Our previous study showed that increasing the phosphorylation levels of the bovine chromaffin cells inhibited the NCX activity assayed in intact cells and isolated plasma membrane vesicles [3]. However, in the present study, activation of PKA

enhanced the exchange activity of the expressed p17h and the endogenous exchange current of chromaffin cells. The reason for the difference is not clear, although the assays for the exchanger activity were different in the two studies. It can be speculated that molecules other than the exchanger might be phosphorylated, thereby affecting the exchange activity. In different assay systems, different molecules might be phosphorylated and exert different effects on the exchanger [26].

Our results of the cardiac clone p17 are consistent with previous studies by Reeves and co-workers. In CHO cells transfected with the bovine cardiac p17 clone, CK1.4 cells, the exchange activity was not affected by PMA. In addition, the same group found no phosphorylation of the exchanger in $^{32}P$-labelled CK1.4 cells [27]. However, when the canine cardiac NCX1 was expressed in CCL39 cells and rat neonatal cardiomyocytes, PKC catalysed the phosphorylation of the N-terminal part of the intracellular loop of the exchanger and up-regulated the exchanger activity [20]. It should be noted that there are no predicted PKC phosphorylation sites in the N-terminal half of the intracellular loop. In a baby hamster kidney cell transfected with canine NCX1, the activation of PKA and PKC moderately enhances the exchange activity [28]. The difference in the effect of activation of PKC on the exchange activity between these studies might arise from the expression systems used. It is therefore possible that the effect of phosphorylation on the activity of the expressed NCX comes from its interaction with other molecules in the cells.

Although the role of phosphorylation in the regulation of the exchanger activity remains to be elucidated, our results provide evidence that alternative splicing of the intracellular loop is important in the regulation of the exchanger activity of different isoforms.

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