Involvement of G protein coupled-receptor kinase 4 and 6 in rapid desensitization of dopamine D₁ receptor in rat IEC-6 intestinal epithelial cells

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**Abstract**

Dopamine-induced inhibition of Na⁺-K⁺-ATPase has been suggested to play a role in the regulation of Na⁺ absorption at the intestinal level and these effects were mediated by dopamine D₁–like receptors. The aim of this work was to evaluate the effect of the activation of the D₁–like receptors on the activity of the Na⁺/H⁺ exchanger (NHE) in the rat intestinal epithelial cell line IEC-6. The presence of D₁ receptors was confirmed by immunoblotting. The dopamine D₁–like receptor agonist SKF 38393 produced a concentration-dependent inhibition of NHE activity and stimulation of adenylyl cyclase (AC), this being antagonized by the D₁ selective antagonist SKF 83566. Effects of SKF 38393 on NHE and AC activities were maximal at 5 min of exposure to the agonist and rapidly diminished with no effect at 25 min. Exposure of cells for 25 min to dibutyrly-cAMP (0.5 mM) or to the AC activator forskolin (3 µM) effectively inhibited NHE activity. Pretreatment of cells with heparin (1 µM), a non-selective G protein coupled-receptor kinase (GRK) inhibitor, prevented the loss of effects on NHE activity after 25 min exposure to SKF 38393. The presence of GRK 4, GRK 6A and GRK 6B was confirmed by immunoblotting. Overnight treatment with the anti-GRK 4-6 antibody complexed with Lipofectin was also effective in preventing loss of the effects of SKF 38393 on NHE and AC activities. It is concluded that dopamine D₁ receptors in IEC-6 rapidly desensitize to D₁-like agonist stimulation and GRK 4 and 6 appear to be involved in agonist-mediated responsiveness and desensitization.

**Key words:** Dopamine – GRK – Na⁺/H⁺ exchanger – adenylyl cyclase - desensitization

**Running title:** GRK 4 and 6 in agonist-induced desensitization of D₁ receptor
Introduction

Dopamine receptors belong to the large family of G protein-coupled receptors and thus far, five distinct genes encoding different dopamine receptor proteins were isolated and characterized in mammals (4, 20, 30). These proteins can be subdivided into D1- and D2-like receptors that differ in their structural, pharmacological and transductional properties (12, 28). The D1-like receptors are generally coupled to Gs and their activation leads to a stimulation of adenylyl cyclase (AC) activity and increased levels of the second messenger cAMP. In jejunal cells, the dopamine inhibitory effect upon Na+-K+-ATPase activity has been shown to be mediated by activation of this subclass of dopamine receptors (33), however this effect was observed in young but not in adult rats (17, 32, 33). This absence of dopamine effect in adult jejunal cells may be explained by regulatory mechanisms that modulate signaling by G protein-coupled receptors such as agonist-induced desensitization of receptors. On the other hand, jejunal epithelial cells from spontaneous hypertensive rats (SHR), in contrast to their normotensive controls (Wistar-Kyoto rats, WKY), fail to respond to dopamine (18), as has been reported for the kidney. In genetic hypertension, the D1 receptor is uncoupled from its G protein complex, resulting in a decreased ability to regulate renal sodium transport. The impaired D1 receptor/G protein coupling in renal proximal tubules in genetic hypertension is secondary to abnormal phosphorylation and desensitization of the D1 receptor (14).

Phosphorylation is often involved in receptor desensitization and is mediated by two classes of serine/threonine kinases: the second messenger-dependent kinases such as protein kinase A and protein kinase C or the specific kinases that phosphorylate the agonist-occupied or activated receptors and referred to as G protein-coupled receptor kinases (GRKs) (8, 15, 26). This family of kinases is composed of seven members (GRK 1 to GRK 7) and is widely expressed which is suggestive of their important role in the regulation of G protein coupled-receptors responsiveness. Involvement of these kinases in agonist-induced desensitization of dopamine D1-like receptors has been previously reported. Indeed, intracellular inhibitors of kinases or elimination of potential phosphorylation sites in the receptors via site-directed mutagenesis could attenuate D1 receptor desensitization (13). Moreover, studies involving heterologously expressed D1 receptors in Sf9 (21), HEK 293 (31) or CHO cells (6) have shown that the D1 receptor undergoes agonist-
induced phosphorylation. In HEK 293 and CHO cells this phenomenon is enhanced by coexpression with GRKs 2, 3, 4 and 5 (6, 31). Recently, in renal proximal tubule cells a role for GRKs 2 and 4 in the homologous desensitization of D1 receptors was reported (6, 34).

The aim of the present study was to evaluate the effect of the activation of the D1–like receptors on the activity of the Na⁺/H⁺ exchanger (NHE) in IEC-6 cells, a rat epithelial cell line that in culture have features of small intestinal crypt cells (27). It is reported that IEC-6 cells are endowed with dopamine D1 receptors, the stimulation of which results in inhibition of NHE activity and AC stimulation. These effects, however, rapidly diminished, but recovered after treatment of cells with the non-specific GRK inhibitor heparin or the anti-GRK 4-6 antibody. The dopamine D1-like-induced NHE inhibition is a cAMP mediated event, but PKA is suggested not to be involved in receptor desensitization.

Methods

Cell culture

IEC-6 cells were obtained from the “Deutsche Sammlung von Mikroorganismen und Zellkulturen” (ACC-111; Passages 3-14) and maintained in a humidified atmosphere of 5% CO₂-95% air at 37°C. Cells were grown in Dulbecco’s Modified Eagle’s Medium (45%) and RPMI 1640 (45%) supplemented with 10% fetal bovine serum, 0.1U/ml insulin, 100 U/ml penicillin G, 0.25 µg/ml amphotericin B and 100µg/ml streptomycin. For subculturing, the cells were dissociated with 0.05% trypsin-EDTA, split 1:4 and subcultured in Costar Petri dishes with 21cm² growth area (Costar, Badhoevedorp, The Netherlands). The cell medium was changed every 2 days and the cells reached confluence 4 days after initial seeding. For 24 hours prior to each experiment, the cell medium was free of fetal bovine serum. Experiments with IEC-6 cells were generally performed 2 days after cells reached confluence; each cm² contained about 20 µg of cell protein.

Na⁺/H⁺ exchanger activity

Na⁺/H⁺ exchanger (NHE) activity was assayed as the initial rate of pHᵢ recovery after an acid load imposed by 20 mM NH₄Cl followed by removal of Na⁺ from the
Krebs’ modified buffer solution (in mM: NaCl 140, KCl 5.4, CaCl₂ 2.8, MgSO₄ 1.2, NaH₂PO₄ 0.3, HEPES 10, glucose 5, pH 7.4) in the absence of CO₂/HCO₃⁻. In these experiments NaCl was replaced by an equimolar concentration of tetramethylammonium chloride (TMA). Intracellular pH measurements were performed in cells cultured in 96 well plates (11). Briefly, cells were loaded in serum-free medium with 5 µM BCECF/AM, the membrane-permeant acetoxyethyl ester derivative of 2',7'- bis (carboxyethyl)-5,6-carboxyfluorescein (BCECF) for 40 min at 37°C in 5% CO₂ - 95% air atmosphere. The cells were washed free of dye and the test compounds added to the extracellular fluid 25, 10 or 5 min before starting the sodium-dependent pH recovery. Cells were placed in the sample compartment of a dual-scanning microplate spectrofluorometer (Spectramax Gemini, Molecular Devices, Sunnyvale, USA) and fluorescence monitored every 17 s alternating between 440 and 490 nm excitation at 535 nm of emission, with a cutoff filter of 530 nm. The ratio of intracellular BCECF fluorescence at 490 nm and 440 nm was converted to pHi by comparison with values from an intracellular calibration curve using the nigericin 10 µM and high-K⁺ method with pH ranging from pH 6.6- pH 7.8.

Effect of GRK inhibitors

In this set of experiments designed to evaluate the role of GRKs in the desensitization mechanism, cells were preincubated overnight at 37°C in 5% CO₂ - 95% air atmosphere in the presence of heparin (1 µM) or monoclonal anti-GRK 4-6 (1.4 µg/mL) (Sigma Chemical Company, St. Louis, MO) together with 5 µg/mL Lipofectin (Invitrogen, San Diego, CA) to facilitate their entry in cells (10, 34).

cAMP measurement

Total cAMP was determined with an enzyme immunoassay kit (Amersham Life, Arlington Heights, IL) according to manufacturer’s protocol. Briefly, IEC-6 cells were pre-incubated for 15 min at 37°C in Hanks’ medium (in mM: NaCl 137, KCl 5, MgSO₄ 0.8, Na₂HPO₄ 0.33, KH₂PO₄ 0.44, CaCl₂ 0.25, MgCl₂ 1.0, TrisHCl 0.15
and sodium butyrate 1.0, pH 7.4) containing 100 µM IBMX (3-isobutyl-1-methylxanthine), a phosphodiesterase inhibitor. Cells were then incubated for 5 to 25 min with increasing concentrations of the D₁-like receptor selective agonist SKF 38393 (0.1-1 µM). The reaction was stopped by addition of 20 µL of lysis reagent (2.5% dodecyltrimethylammonium bromide in 0.05 M acetate buffer pH 5.8 containing 0.02% (w/v) bovine serum albumin) and aliquots were taken for measurement of total cAMP content.

**Western Blotting analysis**

IEC-6 cells cultured to 90% of confluence were washed twice with PBS and total cell protein extracted for dopamine D₁ receptor, NHE1, NHE3, GRK 4, GRK 6A and GRK 6B detection. Briefly, to obtain total cell extract, cells were lysed by brief sonication (15 s) in lysis buffer with protease inhibitors (150mM NaCl, 50 mM Tris-HCl pH 7.4, 5 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 100µg/mL PMSF, aprotinin and leupeptin 2µg/mL each) and incubated on ice for 1 hour. After centrifugation (16,000 g, 30 min, 4ºC), the supernatant was collected and protein concentration determined using the method of Bradford (3). Forty micrograms (NHE1 and NHE3), twenty five micrograms (D₁ receptor) or fifty micrograms of protein (GRK 4, GRK 6A and GRK 6B) were mixed in 6x sample buffer (0.35 M Tris-HCl, 4% SDS, 30% glycerol, 9.3% DTT, pH 6.8, 0.01% bromphenol blue) and boiled for 5 min. Proteins were subjected to SDS-PAGE (10% SDS-polyacrylamide gel) and electrotransfered onto nitrocellulose membranes. The transblot sheets were blocked with 5% of non-fat dry milk in Tris-HCl 25 mM pH 7.5, NaCl 150 mM and 0.1% Tween 20, overnight at 4ºC. Then, the membranes were incubated with appropriately diluted antibodies: rabbit anti-NHE1 or anti-NHE3 polyclonal antibodies (Alpha Diagnostics, Autogenbioclear, Wiltshire, UK), rabbit anti-dopamine receptor D₁A polyclonal antibody (Chemicon® International, Ternecula, CA), rabbit anti-human GRK 4 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-GRK 6A and GRK 6B (35). The reaction was detected by peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and ECL system (Amersham Life, Arlington Heights, IL).
Drugs

Amiloride, forskolin, H-89, low molecular weight heparin, SKF 38393 hydrochloride, SKF 83566 hydrochloride, dibutyryl-cAMP were purchased from Sigma Chemical Company, St. Louis, MO. BCECF-AM, ethylisopropylamiloride (EIPA) and nigericin were obtained from Molecular Probes (Eugene, OR). 4-Isopropyl-3-methylsulfonylbenzoyl-guanidine methanesulfonate (HOE 642) and (3-(54)-N-isopropylidene-2-methyl-acrylamide dihydrochloride (S 3226) were kindly provided by Dr. H. J. Lang from Aventis Pharma Deutschland (Frankfurt, Germany).

Data analysis

Geometric means are given with 95% confidence limits and arithmetic means are given with S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. A P value less than 0.05 was assumed to denote a significant difference. IC50 values were determined from sigmoidal dose-response analysis using the GraphPad Prism statistics software.

Results

In the present study, NHE activity was assayed in IEC-6 cells loaded with a pH-sensitive dye (BCECF), as the Na+-dependent recovery of pH, measured after an acid load imposed by 20 mM NH4Cl followed by removal of Na+ from the Krebs modified buffer solution, in the absence of CO2/HCO3. As shown in figure 1, after acidification, IEC-6 cells showed a rapid alkalinization upon addition of 140 mM Na+. This alkalinization process was largely inhibited by amiloride (1 mM) and by EIPA (10 µM), indicating that the endogenous NHE in IEC-6 cells is both an amiloride- and EIPA-inhibitable exchanger. The sensitivity of NHE to inhibition by amiloride and EIPA was also evaluated. Figure 2A shows inhibition of NHE activity in IEC-6 cells by both amiloride and EIPA, EIPA being considerably more potent than amiloride (IC50 values: amiloride= 54 µM; EIPA= 54 nM). Differences in sensitivity to inhibitors are in agreement with the observation that IEC-6 cells express both the EIPA-sensitive NHE3 subtype and the amiloride-sensitive NHE1...
The presence of both NHE1 and NHE3 isoforms was also confirmed by immunoblotting, as shown in figure 3. Immunoblotting studies also showed that this cell line endogenously expresses dopamine D1 receptors (figure 3). Because D1-like receptors are generally coupled to Gs and their activation leads to a stimulation of AC activity and increased levels of the second messenger cAMP, it was decided to evaluate the effects of cAMP on NHE activity. For this purpose IEC-6 cells were stimulated for 25 min with dibutyryl-cAMP (0.5 mM) or the AC activator forskolin (3 µM). Both compounds markedly reduced NHE activity (forskolin, 28±3 % decrease; dibutyryl-cAMP, 24±4 % decrease).

The activation of dopamine receptors by the selective D1-like dopamine receptor agonist SKF 38393 markedly inhibited NHE activity (figure 4A). This inhibition was maximal at 5 min of exposure to the agonist (30 %) and was progressively lost with the increase of exposure time to the agonist (10 min, 16 % reduction; 25 min, 6 % reduction). Similarly, the selective D1-like dopamine receptor agonist SKF 38393 markedly increased cAMP accumulation, this being most prominent at 5 min of exposure to the agonist (39 %) and was progressively lost with the increase of exposure time to the agonist (10 min, 23 % increase; 25 min, 2 % increase (figure 4B). The maximal inhibition on NHE activity (figure 5A) and the maximal stimulation of cAMP accumulation (figure 5B) obtained after 5 min exposure to SKF 38393 were both concentration-dependent effects and prevented by the selective D1-like dopamine receptor antagonist SKF 83566 (figure 6A and 6B).

The recovery of pHi was clearly a Na+ dependent process in IEC-6 cells and treatment for 5 min with SKF 38393 (1 µM) markedly attenuated the Na+-dependent recovery of pHi. As shown in figure 7, the maximal rate at which the pHi recovery occurred (in pH units/s) in IEC-6 cells treated with 1 µM SKF 38393 (0.0025±0.0003) was lower (P< 0.05) than that in vehicle-treated cells (0.0038±0.0003), with similar K_m values (in mM) for Na+ (vehicle, 14±5; SKF 38393, 10±5). The finding that Na+ removal reduced approximately 90 % of the recovery of pHi, excludes the contribution of the H+-ATPase and clearly demonstrates a specific contribution of NHE activity during the alkalinization process after the Na+ removal.

In order to clarify which isoform of NHE is under control of dopamine D1-like receptor stimulation, specific inhibitors to NHE1 and NHE3 were used. As shown in...
**Discussion**

The results presented here show that rat IEC-6 intestinal epithelial cells are endowed with dopamine D₁ receptors, the stimulation of which results in inhibition of NHE activity and AC stimulation. The use of selective inhibitors of NHE1 and NHE3 isoforms suggest that dopamine D₁-like receptors attenuate the Na⁺-dependent recovery of pHᵢ through inhibition of NHE1. These effects, however,
rapidly diminish, but were perpetuated by treatment of cells with the non-specific GRK inhibitor heparin or the anti-GRK 4-6 antibody. The dopamine D1-like receptor-induced NHE inhibition is a cAMP mediated event, but PKA is probably not to be involved in receptor desensitization.

G protein coupled-receptors can be regulated by desensitization and it is generally accepted that desensitization involves receptor phosphorylation (7, 8). PKA- and PKC-mediated phosphorylation uncouple the receptors from their respective G protein whereas GRK-mediated desensitization is initiated by agonist-induced conformational changes in the receptor that upon phosphorylation becomes a substrate of arrestins, a family of proteins that participates in internalization of the receptors (25). The participation of PKA in dopamine D1 receptor desensitization has been previously reported, though there are some discrepancies. Some authors have shown that intracellular inhibitors of PKA (36) or elimination of potential phosphorylation sites in the receptors via site-directed mutagenesis (13) could attenuate D1 receptor desensitization and that PKA phosphorylation was involved in D1 receptor trafficking (19). In contrast, other authors (2, 16) provided data supporting the view that PKA does not have a relevant role in dopamine D1 receptor homologous desensitization. The present study shows that dopamine D1 receptors in the intestinal epithelial cell line IEC-6 rapidly desensitize to D1-like stimulation and increases in cAMP or activation of PKA are not responsible for desensitization. In fact, increases in intracellular cAMP following stimulation with D1-like agonist SKF 38393 attained its maximum at 5 min exposure to the agonist and rapidly dissipated. On the other hand, exposure of cells for 25 min with the membrane-permeable cAMP analog dibutyryl-cAMP or with the AC activator forskolin effectively inhibited NHE activity in IEC-6 cells.

GRKs are serine-threonine protein kinases that are implicated in homologous desensitization of a variety of GPCRs (1, 5, 9, 29). Since GRKs are known to participate in dopamine D1 receptor homologous desensitization (31, 34), it was decided to determine their involvement in this desensitization process in IEC-6 cells. Heparin, a non-specific GRK inhibitor (13), prevented the loss of D1-like dopamine receptor-mediated inhibition of NHE activity after 25 min of exposure to SKF 38393 inhibition. The GRK family includes 7 members that are divided in three groups according to their localization and functional domains: GRK 1 and 7, GRK 2 and 3, and GRK 4, 5 and 6 (7, 24). Recently, GRK 4 has been reported to
play a critical role in the homologous desensitization of D1 receptors in renal proximal tubule cells (34). Immunoblotting showed that IEC-6 cells endogenously express GRK 4, GRK 6A and GRK 6B. Functional studies were also done and cells treated overnight with the anti-GRK 4-6 antibody before assessment of NHE activity. Treatment of cells with the anti-GRK 4-6 antibody was effective in preventing the loss of D1-mediated inhibitory effect on NHE activity. In addition, treatment with the anti-GRK 4-6 antibody also prevented the loss of effect of SKF 38393 on AC stimulation, as evidenced by similar increases in cAMP formation after 5 and 25 min exposure to SKF 38393. This clearly indicates that desensitization of dopamine D1-like receptor might be not a PKA-mediated event. However, the dopamine D1-like receptor-mediated NHE inhibition is a PKA-mediated event, as evidenced by the prevention of these effects by the PKA inhibitor H-89.

In conclusion, sustained dopamine D1-like receptor stimulation in IEC-6 cells, a rat intestinal epithelial cell line that expresses the dopamine D1 receptor, results in rapid desensitization, as evidenced by decreases in NHE inhibition and AC stimulation. Desensitization of both post-receptor events, inhibition of NHE activity and stimulation of AC activity, is suggested to occur as a result of GRK-mediated phosphorylation of dopamine D1-like receptors, namely those belonging to the GRK 4 and 6 subgroup.

Acknowledgments

SF is supported by grant SFRH/BD/4595/2001 from Fundação para a Ciência e a Tecnologia (Portugal). This study was supported in part by grant HL23081 from the National Institutes of Health (USA).
References


Legends to figures

**Figure 1.** Assessment of intracellular pH (pH_i) during the Na⁺-dependent pH_i recovery after an acid load imposed by exposure to NH₄Cl followed by Na⁺ removal of the incubation medium in IEC-6 cells, in the absence and the presence of EIPA (10 µM) or amiloride (1 mM). Symbols represent means of 8 experiments per group; vertical lines show S.E.M.

**Figure 2.** Effect of EIPA (0.01-10 µM) and amiloride (0.1-1 mM) on Na⁺/H⁺ exchanger activity in IEC-6 cells. IC₅₀ values with intervals of 95% of confidence. Symbols represent the mean of 8 experiments per group; vertical lines show S.E.M.

**Figure 3.** Immunoblots of isoform 1 and 3 of Na⁺/H⁺ exchanger (panel A), GRK 4 and GRK 6A and GRK 6B (panel B), dopamine D₁ receptor (panel C) of total cellular lysates from IEC-6 cells separated on SDS-PAGE 10%, transferred to a nitrocellulose filter and performed using specific antibodies. Bands were detected by the ECL method. Arrows indicate the specific bands of about 90 kDa for NHE1 and NHE3, 65 kDa for GRK 4, GRK 6A and GRK 6B and 50 kDa for D₁ receptor.

**Figure 4.** Effect of SKF 38393 (1 µM) for 5, 10 and 25 min on (A) the Na⁺/H⁺ exchanger activity and (B) total cAMP content in IEC-6 cells. Columns represent the mean of 7 to 31 experiments per group; vertical lines show S.E.M.. Significantly different from the respective control value (* P<0.05).

**Figure 5.** (A) Na⁺/H⁺ exchanger activity and (B) total cAMP content in IEC-6 cells treated with vehicle and increasing concentrations of SKF 38393 (5 min exposure). Columns represent the mean of 3 to 43 experiments per group; vertical lines show S.E.M.. Significantly different from the control value (* P<0.05).

**Figure 6.** Effect of dopamine D₁-like receptor antagonist SKF 83566 (1 µM) on (A) Na⁺/H⁺ exchanger activity and (B) total cAMP content in IEC-6 cells under basal or after 5 min exposure to SKF 38393 (1 µM). Columns represent the mean of 3 to 4
experiments per group; vertical lines show S.E.M. Significantly different from control values (* P<0.05).

**Figure 7.** Na⁺ dependence of Na⁺/H⁺ exchanger activity in vehicle and SKF 38393 (1 µM, 5min exposure)-treated IEC-6 cells. Each point represents the mean of 6 experiments; vertical lines indicate S.E.M.

**Figure 8.** Effect of NHE1 specific inhibitor HOE 642 (0.3 µM) (A) and NHE3 specific inhibitor S3226 (0.3 µM) (B) on Na⁺/H⁺ exchanger activity in IEC-6 cells under basal or after 5 min exposure to SKF 38393 (1 µM). Columns represent the mean of 14 to 31 experiments per group; vertical lines show S.E.M. Significantly different from the control value (* P<0.05).

**Figure 9.** Effect of SKF 38393 (1 µM, for 25 and 5 min of exposure) on Na⁺/H⁺ exchanger activity in IEC-6 cells treated overnight with (A) the non-selective GRK inhibitor heparin (1 µM) and (B) the anti-GRK 4-6 antibody (1.4 µg/mL). Columns represent the mean of 7 to 15 experiments per group; vertical lines show S.E.M. Significantly different from the corresponding control values in vehicle-treated cells (* P<0.05).

**Figure 10.** Effect of overnight pre-treatment with anti-GRK 4-6 antibody (1.4 µg/mL) on total cAMP content under basal and SKF 38393 (1 µM; 25 and 5 min exposure)-stimulated IEC-6 cells. Columns represent the mean of 6-10 experiments per group; vertical lines show S.E.M. Significantly different from control values (* P<0.05).

**Figure 11.** Prevention of agonist-induced inhibition (SKF 38393 1 µM; 25 min exposure) of Na⁺/H⁺ exchanger activity in anti-GRK 4-6 antibody (1.4 µg/mL) pre-treated IEC-6 cells by the PKA inhibitor H-89 (10µM). Columns represent the mean of 7-17 experiments per group; vertical lines show S.E.M. Significantly different from control values (* P<0.05).
Fig 1

![Graph showing ∆pH over time for different conditions: Control, Amiloride (1 mM), EIPA (10 µM).](image-url)

- Control
- Amiloride (1 mM)
- EIPA (10 µM)
Fig 2

A

\[ \text{Na}^+ / \text{H}^+ \text{ activity (}\% \text{ of control)} \]

\[ \text{IC}_{50} = 0.054 (0.022-0.130) \text{ µM} \]

B

\[ \text{Na}^+ / \text{H}^+ \text{ activity (}\% \text{ of control)} \]

\[ \text{IC}_{50} = 53.7 (12.8-224.2) \text{ µM} \]
Fig 4

A

![Graph A showing Na+/H+ exchanger activity (% of control)]

- Control
- SKF 5′
- SKF 10′
- SKF 25′

B

![Graph B showing cyclic AMP (fmol/well)]

- Control
- SKF 5′
- SKF 10′
- SKF 25′
Fig 5

A

[Bar graph showing Na+/H+ exchanger activity (% of control) with SKF 38393 (µM) on the x-axis.]

B

[Bar graph showing cyclic AMP (% of control) with SKF 38393 (µM) on the x-axis.]
Fig 6

A

Na+/H+ exchanger activity (% of control)

B

cyclic AMP (% of control)
Fig 7

Na⁺/H⁺ exchanger activity

[Na⁺] mM
**Fig 9**

**A**

Vehicle 25 min 5 min

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**B**

Vehicle 25 min 5 min

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Fig 10

![Bar graph showing cyclic AMP levels in Vehicle and GRK 4-6 samples.](image)

- Basal
- SKF 5'
- SKF 25'

Cyclic AMP (% of control)
Fig 11

![Bar chart showing Na⁺/H⁺ exchanger activity (% of control) for different treatments. The x-axis represents Vehicle and SKF 38393, while the y-axis represents activity. The treatments are differentiated by bars in white, black, and gray, indicating Control, anti-GRK, and anti-GRK + H-89, respectively. The chart includes error bars indicating variability.](image-url)