Regulation of the Na\(^+\)/K\(^+\)-ATPase Pump *in Vitro* after Long-Term Exposure to Cocaine: Role of Serotonin\(^1\)

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**ABSTRACT**

Long-term exposure to cocaine can cause persistent behavioral changes and alterations in neuronal function. One cocaine-regulated mRNA in the rat brain is the beta-1 subunit of the Na\(^+\)/K\(^+\)-ATPase pump. We examined both Na\(^+\)/K\(^+\)-ATPase function and expression after cocaine treatment of pheochromocytoma cells. One-hour exposure to cocaine did not alter Na\(^+\)/K\(^+\)-ATPase activity, as measured by the ouabain-sensitive component of rubidium uptake. Four days of cocaine resulted in an ~30% decrease in Na\(^+\)/K\(^+\)-ATPase activity. Western blot analyses demonstrated an ~25% decrease in levels of the beta-1 isoform, without changes in pump total alpha subunit levels. Treatment with dopamine type 1 or type 2 receptor agonists for the same period did not affect Na\(^+\)/K\(^+\)-ATPase activity. The serotonin-selective reuptake inhibitor paroxetine caused an ~45% decrease in rubidium uptake after 4 days, whereas pump function was not altered after treatment with either the dopamine-selective reuptake blocker nomifensine or the norepinephrine-selective reuptake blocker desipramine. Chronic treatment with both cocaine and LY 278,584, a serotonin type 3 receptor antagonist, did not replicate the cocaine-associated decrease in pump function. Long-term cocaine exposure regulates expression and function of the Na\(^+\)/K\(^+\)-ATPase pump in neuronal-like cells; this regulation is mediated in part via the serotonin type 3 receptor. Similar Na\(^+\)/K\(^+\)-ATPase pump regulation *in vivo* may selectively alter neuronal function in the mammalian brain.

Repeated exposure to cocaine can result in persistent changes in neuronal function (Pierce et al., 1996; White et al., 1995). Rats also may exhibit a sensitized locomotor response to a single dose of cocaine after several days of prior cocaine treatment (Kalivas and Stewart, 1991; Robinson and Becker, 1986). The molecular mechanisms that mediate these biochemical and behavioral events are not well understood; however, selective changes in neuronal gene expression are thought to contribute to some of the long-term functional alterations (Mackler and Eberwine, 1992; Nestler et al., 1993). Changes in the levels of specific mRNAs have been observed after repeated cocaine treatment (Graybiel et al., 1990; Moratalla et al., 1996; Nestler et al., 1993) or withdrawal after cocaine treatment (Cha et al., 1997; Moratalla et al., 1996). A differential hybridization approach was performed with cDNAs isolated from the nucleus accumbens of rats 3 weeks after withdrawal from cocaine self-administration to isolate cocaine-regulated mRNAs. Initial results identified the beta-1 isoform of the Na\(^+\)/K\(^+\)-ATPase pump as one mRNA regulated several weeks after cocaine self-administration (Cha et al., 1996). This finding helped support the hypothesis that altered Na\(^+\)/K\(^+\)-ATPase pump activity may lead to increases in extracellular glutamate levels in the nucleus accumbens of sensitized rats (Pierce et al., 1996). Therefore, detailed studies examining the effects of cocaine on the Na\(^+\)/K\(^+\)-ATPase pump are warranted. Treatment of pregnant guinea pigs with cocaine resulted in a decrease in fetal brain Na\(^+\)/K\(^+\)-ATPase pump activity (Lien et al., 1994); otherwise, interactions between cocaine and the Na\(^+\)/K\(^+\)-ATPase pump have not been reported previously. The molecular consequences of cocaine treatment include the inhibition of biogenic amine reuptake (Ritz et al., 1987) and blockade of the voltage-sensitive Na\(^+\) channel (Hille, 1967). Dopamine reuptake blockade correlated best with the self-administration of cocaine (Ritz et al., 1987); however, cocaine’s other actions, including inhibition of the reuptake of 5-HT and its property as a local anesthetic, also may contribute to the regulation of different mRNAs that demonstrate altered levels after cocaine treatment.

Pheochromocytome 12 cells (Greene and Tischler, 1976) and various PC12 subclones can be differentiated into a postmitotic state that exhibits many of the biochemical, morphological and electrophysiological features of neurons (Inoue et al., 1988; Pittman et al., 1993). This differentiation, induced by exposure to NGF, included increases in mRNA

**ABBREVIATION:** ECL, enzymatic chemiluminescence; 5-HT, serotonin; NGF, nerve growth factor; PC, pheochromocytoma; Rb\(^+\), rubidium; HEPES, N-2-hydroxyethylpiperazine-N\(^\prime\)-2-ethanesulfonic acid.
and protein levels for the dopamine transporter along with a concurrent decrease in mRNA levels for the norepinephrine transporter (Kadota et al., 1996). Cocaine blocked dopamine uptake in both undifferentiated and differentiated PC cells (Kadota et al., 1996; Zhu and Hexum, 1992). Cocaine at submicromolar concentrations had little effect on the viability and differentiation of PC 12 cells (Zachor et al., 1994). PC 12 cells are thus a suitable in vitro system for studying the biochemical and physiological effects of cocaine exposure on monoaminergic neurons. The present study investigated whether or not daily cocaine treatment for several days regulates both the function and levels of the Na+/K+-ATPase pump in the PC 6–3 subclone (Pittman et al., 1993) of PC12 cells.

Materials and Methods

Tissue culture and drug treatments. PC 6–3 cells were maintained in complete media (RPMI media with 10% horse serum, 5% fetal calf serum, 100 units/ml penicillin and 0.1 mg/ml streptomycin) at 37°C in a 5% CO₂, humidified incubator (Pittman et al., 1993). Cells with passage numbers 2 to 8 were seeded at a density of ~30 to 40% onto collagen-adsorbed (Collagen Corp., Palo Alto, CA) culture plates, and differentiated into their neuronal phenotype by the addition of NGF (100 ng/ml; Collaborative Research, Bedford, MA) for 3 days before further pharmacologic treatment. On each day of subsequent drug treatment, ~50% of the media was removed and replaced with media that contained NGF and the specified drug. Drugs were purchased from RBI (Natick, MA) (SKF-38393 HCl, quinpirole HCl, desipramine HCl, methysergide maleate and LY 278,584 maleate) or Sigma (St. Louis, MO) (ouabain). Cocaine HCl was supplied by the National Institute on Drug Abuse, and paroxetine was a gift from Dr. Irwin Lucki (Univ. of Pennsylvania). Cocaine doses were selected based on both the Kᵣ for cocaine-sensitive dopamine uptake in PC cells (Zhu and Hexum, 1992) and serum levels observed in human volunteers (Foltin and Fischman, 1991). Cocaine levels in the media before and after daily drug treatments were determined in a subset of experiments by fluorescence polarization immunoassay (Perez-Bendito et al., 1994).

Rubidium uptake assays. The ouabain-sensitive component of total Rb⁺ uptake was measured at 22–24°C and used to calculate Na+/K¹-ATPase pump activity (Inoue et al., 1988). PC 6–3 cells were washed three times with Rb⁺-buffer (130 mM NaCl, 0.5 mM RbCl, 1.8 mM CaCl₂, 0.8 mM MgCl₂, 5 mM glucose and 5 mM HEPES (pH 7.4)) and preincubated in 0.2 ml of the same buffer with or without 5 mM ouabain for 30 min. Ten microliters of buffer that included 0.4 μl of [66Rb]Cl (specific activity, 10 μCi/μl; New England Nuclear, Boston, MA) were added to each well, the cells incubated for 9 min, and uptake terminated by removal of the buffer followed by two washes in ice-cold buffer. The cells were lysed by the addition of 0.2 ml of 1 N NaOH, and collected into Eppendorf tubes that already contained 0.2 ml of 1 N HCl. Wells were washed with 0.2 ml of H₂O, and this volume also was added to each Eppendorf tube. The amount of radioactivity in each tube was measured by Cerenkov radiation, and then 50 to 100 μl used to determine the total protein amount for each sample (Bradford, 1976). The ouabain-sensitive Rb⁺ uptake was calculated as the total cpm/μg protein/9-min incubation minus the ouabain-insensitive cpm/μg protein/9-min incubation. Each experiment, with or without ouabain, included four wells per treatment and the averages for each quadruplicate set used as a final value. A minimum of three experiments were completed for each treatment group.

Western blot analysis. Total protein was isolated from PC 6–3 cells in solubilization buffer with protease inhibitors after the indicated treatment periods for each experiment. Equal amounts of total protein (30–50 μg) were heat-denatured, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5% polyacrylamide) and transferred to nitrocellulose blots. Na+/K¹-ATPase pump subunit levels were detected by ECL with streptavidin-conjugated alkaline phosphatase, according to the manufacturer’s instructions (Tropix, Inc., Bedford, MA). A polyclonal antibody directed against the mammalian beta-1 subunit of the Na+/K¹-ATPase pump was purchased from UBI, Inc. (Lake Placid, NY), and a polyclonal antibody that recognizes all three alpha subunits of the Na+/K¹-ATPase pump was supplied by Dr. Stephen Ernst (University of Michigan). Both polyclonal antibodies were used at 1:1000 dilutions. Separate lanes with identical amounts of total protein were handled in a similar manner for ECL, but did not include incubation with the primary antibody. Intensities of the specific bands, at the appropriate sizes and not present in the lanes incubated without a primary antibody, were measured with a scanning densitometer (Molecular Dynamics, Inc., Sunnyvale, CA), and mean values were calculated for each treatment group as a percentage of the mean value obtained with untreated cells.

Data analysis. Results for each treatment group were calculated as a percentage of the mean value obtained with untreated cells in the same experiments. Mean values were calculated for each set, and the treatment groups were compared with the untreated groups. Rb⁺ uptake for each drug dose and treatment was performed with parallel control cells; comparisons were made between a drug treatment group and untreated control cells by a two-tailed student’s t test.

Results

Short-term cocaine exposure (1 hr) did not affect Na+/K¹-ATPase pump function in PC 6–3 cells. Initial studies demonstrated that the uptake of Rb⁺ into differentiated PC 6–3 cells was linear from 5 to 10 min, with the assay conditions outlined above (data not shown). Ouabain (5 mM) was required to decrease ≥60% of total Rb⁺ uptake, similar to the conditions described previously (Inoue et al., 1988). Cocaine treatment of differentiated PC 6–3 cells for 1 hr did not result in changes in the ouabain-sensitive component of Rb⁺ uptake (fig. 1). The amount of ouabain-insensitive Rb⁺ also was not altered by cocaine pretreatment (data not shown). These results demonstrate that a short-term exposure to cocaine does not alter Na+/K¹-ATPase pump function in PC 6–3 cells.

Long-term cocaine exposure (4 days) decreased Na+/K¹-ATPase pump function and levels in PC 6–3 cells. Cocaine treatment of PC 6–3 cells for 4 days resulted in a significant decrease in the ouabain-sensitive component of Rb⁺ uptake when compared with untreated cells (fig. 2), without changes in the ouabain-insensitive component of Rb⁺ uptake. This reduction in Na+/K¹-ATPase pump function occurred with several concentrations of cocaine; 0.1 μM [69 ± 10% (S.E.M.), P < .001]; 1 μM (74 ± 12%, P < .05) and 10 μM (62 ± 14%, P < .05). Treatment with 0.01 μM cocaine did not result in a significant change in Na+/K¹-ATPase pump activity. To determine whether this altered activity resulted partly from a decrease in levels of the subunits of the Na+/K¹-ATPase pump protein, Western blots from similarly treated cells were analyzed (figs. 3 and 4). There was no demonstrable change in the total amount of alpha subunit protein (figs. 3A and 4A); however, there was an ~25% reduction in beta-1 subunit levels (figs. 3B and 4B). The decreases in beta-1 subunit protein occurred with the same doses of cocaine that caused similar decrements in Na+/K¹-ATPase pump function (figs. 2 and 4B). A similar dose of another local anesthetic, lidocaine, did not affect beta-1 sub-
not affect ouabain-sensitive Rb⁺ uptake (fig. 5). These data show that cocaine regulation of the Na⁺/K⁺-ATPase pump does not result from the effects of long-term dopamine receptor activation or reuptake blockade.

Selective blockade of serotonin reuptake for 4 days decreased Na⁺/K⁺-ATPase pump function. Cocaine also blocks the reuptake of other biogenic amines (Ritz et al., 1987). Experiments next used 4 days of daily treatment with desipramine or paroxetine at doses that cause selective noradrenergic [desipramine (Koide et al., 1986)] or serotonin [paroxetine (Mathis et al., 1992)] reuptake blockade. Desipramine did not affect Na⁺/K⁺-ATPase activity, but paroxetine decreased pump function by ~45% (fig. 7), which indicates that inhibition of serotonin reuptake into PC 6–3 cells was associated with a significant decrease in Na⁺/K⁺-ATPase pump activity. Western blot analysis showed a decrease in beta-1 subunit levels in response to paroxetine similar to that observed after long-term cocaine treatment (data not shown).

Na⁺/K⁺-ATPase pump function after both long-term cocaine and serotonin receptor antagonist treatment. The decrease in ouabain-sensitive Rb⁺ uptake caused by 4 days of paroxetine treatment suggested that chronic serotonin receptor activation may mediate altered Na⁺/K⁺-ATPase function that was observed with long-term cocaine exposure. PC 6–3 cells were treated with both 0.1 μM cocaine (which causes an ~30% reduction in pump function, fig. 2) and the serotonin receptor antagonists methysergide or LY 278,584. Doses of methysergide that block the metabotropic 5-HT₂/5-HT₃ receptors (Oelszner, 1980) did not reverse the decrease in Na⁺/K⁺-ATPase function associated with cocaine (fig. 8). Concurrent treatment with both LY278,584, an antagonist of the ligand-gated 5-HT₃ cation channel (Kᵢ = 0.66 nM (Wong et al., 1989)), and cocaine for 4 days did prevent the cocaine-associated reduction in Na⁺/K⁺-ATPase function (fig. 8). Daily treatment with either methysergide or LY 278,584 alone for 4 days did not significantly alter Na⁺/K⁺-ATPase activity (data not shown).

Discussion

The present study demonstrates that several days of exposure to cocaine resulted in a decrease in activity of the Na⁺/K⁺-ATPase pump in PC 6–3 cells. This reduction in pump function was accompanied by a decrease in beta-1 subunit protein levels without changes in total alpha₁ subunit expression. The selective serotonin reuptake inhibitor paroxetine also caused a marked reduction in Na⁺/K⁺-ATPase pump activity after a similar time of treatment. Blockade of the ligand-gated 5-HT₃ cation channel reversed cocaine’s ability to alter pump function. Either enhancement of dopaminergic input or lidocaine treatment did not result in similar findings. These experiments indicate that the effects of long-term cocaine treatment on the Na⁺/K⁺-ATPase pump in neuronal-like, PC 6–3 cells are mediated partly by modulation of serotonergic transmission. Daily addition of cocaine was selected to best simulate the intermittent use of cocaine in animal models; the observed reduction in cocaine levels in the media demonstrated that the cells were not exposed continually to high cocaine concentrations.

Interactions between cocaine and 5-HT, and their combined effects on neuronal function, have not been reported.
to the same extent as those effects mediated by the actions of cocaine on dopaminergic pathways. 5-HT has been shown to inhibit \(\text{Na}^+/\text{K}^-\text{ATPase} \) function; both indirectly, by phosphorylation of the pump via 5-HT<sub>2c</sub> receptor activation in the choroid plexus of the rat (Fisone et al., 1995), and directly, after addition to isolated \(\text{Na}^+/\text{K}^-\text{ATPase} \) pump protein from the guinea pig kidney (Stepp and Novakoski, 1997). In contrast, the addition of 5-HT to isolated tissues from several regions of the rat central nervous system increased pump function (Hernandez, 1987), although the 5-HT receptor(s) that mediated these increases were not identified. The types of receptors present apparently are critical to the regulation of pump function by 5-HT. In PC cells, multiple 5-HT receptors are present, including 5-HT<sub>1</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> subtypes (Isenberg et al., 1993). The ligand-gated cation channel 5-HT<sub>2</sub> receptor increased in both amount and function after NGF treatment (Isenberg et al., 1993). Because of this predominance of 5-HT<sub>3</sub> receptors in differentiated PC cells, along with the movement of cations thru these ligand-gated ion channels, the 5-HT<sub>3</sub> receptor may be critical to the cocaine-regulation of the \(\text{Na}^+/\text{K}^-\text{ATPase} \) pump. This premise was supported by the results of this study (fig. 8). All the previous studies have examined the \(\text{Na}^+/\text{K}^-\text{ATPase} \) pump after brief (less than 30 min) exposures to 5-HT, which differs from the present results (figs.1 and 2).
tered endogenous 5-HT levels may be a critical factor in cocaine regulation of the Na⁺/K⁺-ATPase pump cells, because PC cells are able to synthesize 5-HT (Vandenbergh et al., 1991) and transport 5-HT back into the cell (Nakanishi et al., 1995).

The mechanism(s) by which long-term cocaine exposure alters both Na⁺/K⁺-ATPase activity and levels of the β1 subunit do not appear at all to involve dopamine transport or dopamine receptor activation, although modulation of dopaminergic input has been accepted as the predominant mechanism by which cocaine alters function in the mammalian central nervous system (Ritz et al., 1987). The lack of an effect after treatment with either the D1 or D2 agonists (figs. 5 and 6) may have resulted from a rapid inactivation of these drugs by PC 6–3 cells. However, these data, when combined with a similar finding after the use of nomifensine (fig. 5), do not support a role for dopaminergic transmission on cocaine regulation of the Na⁺/K⁺-ATPase pump in PC 6–3 cells. In

**Fig. 4.** The effects of long-term cocaine treatment on Na⁺/K⁺-ATPase pump subunit levels. (top) The densitometric value of the ECL signal for the alpha subunit in each sample was measured by a scanning densitometer and calculated as a percentage of the mean value for untreated cells. No significant differences were observed between control PC 6–3 cells (100 ± 7%) compared with samples from 1 μM (98 ± 6%), 10 μM (92 ± 5%) and 100 μM (94 ± 9%) cocaine. (bottom) Four days of cocaine treatment lead to significant decreases in the amounts of the β1 subunit after exposure to 1 μM (72 ± 3%), 10 μM (76 ± 8%) or 100 μM cocaine (76 ± 8%). Treatment for the same time period with another local anesthetic, lidocaine at 10 μM, did not alter levels of the β1 subunit. *P < .05.
contrast, cocaine's effects on serotonergic neurotransmission may have critical influences on cellular physiology.

Chronic cocaine exposure at 0.01 μM, a dose several orders of magnitude below the $K_i$ for the biogenic amine transporters (e.g., Zhu and Hexum, 1992), did not significantly alter Na$^+/K^+$-ATPase pump activity (fig. 3). The remaining doses

Fig. 5. Enhancement of dopaminergic input does not alter Na$^+/K^+$-ATPase pump function. PC 6–3 cells were exposed to agonists for either the D1 (SKF-38393) or D2 (quinpirole) dopamine receptor subtypes or to the dopamine-reuptake blocker nomifensine daily for 4 days. The drug doses selected for use were those doses that would selectively and maximally affect their target proteins. Neither long-term agonist treatment nor dopamine reuptake blockade significantly affected the ouabain-sensitive component of Rb$^+$ uptake.

Fig. 6. Long-term treatment with dopamine agonists does not change beta-1 subunit levels. Western blots were analyzed after long-term treatment with either SKF-38393 (D1 dopamine receptor subtype agonist) or quinpirole (D2 dopamine receptor subtype agonist) to determine whether the reduction in beta-1 subunit levels observed after several days of cocaine treatment (fig. 5) was present only when a decrease in Na$^+/K^+$-ATPase activity occurred (fig. 2). No significant changes in beta-1 subunit protein amounts were observed in response to dopamine receptor agonist treatment.
did demonstrate decreased Na\(^+\)/K\(^-\)-ATPase pump activity, although not in a linear fashion. This may reflect the fact that several steps contribute to regulation of the Na\(^+\)/K\(^-\)-ATPase pump, from 5-HT reuptake inhibition by cocaine to 5-HT\(_3\) receptor activation and involvement of multiple potential signal transduction pathways. Cocaine doses greater than 10 \(\mu\)M were not used in this study, because these doses would be expected to block the voltage-sensitive Na\(^+\) channels (Hille, 1967) and adversely affect overall cellular function (Zachor et al., 1994).

The cocaine-associated reduction in Na\(^+\)/K\(^-\)-ATPase function (fig. 2) may be caused by several factors, including a decrease in the total number of Na\(^+\)/K\(^-\)-ATPase pump molecules and/or covalent modifications that affect pump function. The present results clearly do not demonstrate how activation of the 5-HT\(_3\) receptor regulates the Na\(^+\)/K\(^-\)-ATPase pump. A decrease in total alpha subunit levels does not explain the reduction in pump activity, because Western blot analysis did not demonstrate any quantitative changes (fig. 4). An antibody that recognizes all three alpha subunits was selected for use in the present study, because the ouabain-sensitive component of Rb\(^-\) uptake results from the sum of total alpha subunit activity and it is therefore important to measure total alpha subunit amounts. The decrease in beta-1 subunit levels was similar in magnitude to the observed reduction in pump activity after long-term cocaine treatment.
(figs. 2 and 4B). This apparent discordant regulation between alpha and beta subunit expression may help to explain the observed decrease in pump activity, because the beta subunits appear to be critical for insertion of the mature Na+/K+-ATPase pump into the plasma membrane (Fambrough et al., 1994; Hiatt et al., 1984). Discordant regulation of alpha and beta subunit mRNA expression has been described in other tissues (Pressley et al., 1988; Yamamoto et al., 1993). Biotinylination might help to determine whether the cocaine-associated decrease in Na+/K+-ATPase function results in part from a disruption of pump insertion into the plasma membrane because of discordant regulation between alpha and beta1 protein expression; but this type of experiment with PC 6–3 cells has been unsuccessful to date. Translational or post-translational changes, including covalent modifications of the Na+/K+-ATPase pump, also may contribute to diminished pump function. Phosphorylation via activation of specific 5-HT receptors is one likely possibility (Fisone et al., 1995). 5-HT treatment of P12 cells for a brief period also caused induction of TISS8/egr-1 and c-fos, both of which are immediate early genes (Humblot et al., 1997). The consequences of treating PC cells with 5-HT therefore include alterations in gene transcription; however, the identities of the genes affected by induction of TISS8/egr-1, c-fos and possibly other regulators of transcription are not known. The increase in these two immediate early genes was associated with protein tyrosine kinase activity, which further suggests that the amount of phosphorylation of the Na+/K+-ATPase pump may be altered by exposure to 5-HT. Finally, both the Na+/K+-ATPase pump and the serotonin transporter exhibited cell sorting similar to the basolateral membranes in transfected epithelial cells (Gu et al., 1996). The co-localization of these two membrane proteins in studies of polarized cells may indicate that direct interactions occur between them, but this would not entirely explain the effects of cocaine on the Na+/K+-ATPase pump in the present study.

An overall toxic effect of cocaine on cellular function apparently does not cause the decrease in Na+/K+-ATPase activity, for at least two reasons. First, there were no significant differences in total protein amounts after treatment of PC 6–3 cells with any of the different drug regimens, compared with untreated cells. Furthermore, there were no obvious changes in neuronal morphology in differentiated PC 6–3 cells after any of the drug treatments (data not shown). This finding is different from a previous study that described maximal reductions in neurite number and complexity after 3 days of exposure to 3 and 30 μM cocaine (Zachor et al., 1994); the effects of cocaine on neuronal morphology may occur at doses greater than those which alter Na+/K+-ATPase function.

The consequences, after long-term cocaine treatment, of reduced Na+/K+-ATPase activity on neuronal function need to be examined. Cocaine or paroxetine may lead to increased intracellular Na+ concentrations after chronic exposure because of a decrease in removal of cytosolic Na+ by the Na+/K+-ATPase pump. However, it is also possible that the decrease in Na+/K+-ATPase pump function is a compensatory response to a reduced intracellular Na+ concentration arising from another effect of long-term drug treatment. Measurement of the intracellular Na+ content should help to support either of these hypotheses. Several days of treatment with another addictive drug, ethanol, did cause increases in intracellular Na+ levels in PC12h cells (Rabin and Acaia, 1993). The elevated Na+ levels, along with decreases in intracellular K+ levels, were associated with enhanced Na+/K+ ATPase pump function, which suggests that altered pump activity was in response to intracellular cation content. If chronic cocaine treatment does alter the intracellular Na+ concentration of neurons, then changes in the transmembrane Na+ potential may affect several facets of neuronal function dramatically, including cellular volume, neuronal excitability or the transport of other molecules via Na+ dependent transport proteins. One hypothesis that may help to explain some of the long-term biochemical and behavioral outcomes of repeated cocaine use is a decrease in the uptake of the excitatory neurotransmitter glutamate in the nucleus accumbens (Pierce et al., 1996). This altered glutamate uptake could arise from a lower Na+ electrochemical gradient because of the Na+-dependence of the glutamate transporters (Robinson et al., 1993).

In summary, long-term treatment of PC 6–3 cells with cocaine resulted in decreased Na+/K+-ATPase activity, a change that was mediated in part by the ligand-gated cation channel 5-HT3 receptor. This reduction in Na+/K+-ATPase function almost certainly will affect cellular physiology. Experiments are now in progress to determine the significance of these changes that occur after long-term cocaine or paroxetine treatment. Similar regulation of Na+/K+-ATPase function may occur in the nucleus accumbens, because 5-HT3 receptors are found in many axons (Pristyle et al., 1997) of this region which are critical to behaviors associated with cocaine use.

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