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Cocaine induces striatal c-Fos-immunoreactive proteins via dopaminergic D_1 receptors

(Fos-related antigens/protooncogenes/transcription factor AP-1/caudate-putamen/nucleus accumbens)

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ABSTRACT **The protooncogene c-*fos* produces a phosphoprotein, Fos, which regulates gene transcription processes. In neuronal systems, Fos has been proposed to couple synaptic transmission to changes in gene expression by acting in the cell nucleus in concert with other proteins to form complexes in the promoter regions of target genes. We report here that the acute administration of a single dose of the indirect-acting dopaminergic agonist cocaine increases multiple Fos proteins in rat caudate nucleus. The increase is dose-dependent and is apparent immunocytochemically at 1 hr, maximal at 2 hr, and absent 48 hr after treatment. The increase seen immunocytochemically is composed of several molecular weight species as assessed by Western blotting of proteins from isolated striatal cell nuclei. Administration of the specific dopaminergic receptor antagonists sulpiride and SCH-23390 prior to cocaine support a significant role for D_1 but not for D_2 receptors in mediating this effect. These data indicate that D_1 dopamine receptors are linked to a cellular immediate-early gene system(s) and suggest an action of cocaine at one or more levels of gene expression via modulation of transcriptional processes in activated cells.**

Cocaine is a psychomotor stimulant that has complex effects in the central nervous system. It acts primarily to block re-uptake of dopamine at the synapse (1–3) but also blocks re-uptake of serotonin (3, 4) and norepinephrine (2, 3, 5). Many of the behavioral effects of cocaine, including its activation of locomotor and stereotypic behaviors, have generally been attributed to its actions as an indirect-acting dopaminergic agonist (6–8). The striatum, which is densely innervated by dopaminergic fibers arising from the substantia nigra and ventral tegmental area, is considered an important neural substrate of these behavioral effects (9). Within the basal ganglia, dopaminergic neurotransmission can influence various sets of neuronal perikarya that synthesize several neuropeptides, including substance P and the opioid peptides enkephalin and dynorphin (10). Treatment with dopaminergic receptor agonists and antagonists has profound and highly specific effects on neuropeptide biosynthesis in the basal ganglia. For example, chronic drug-induced activation of the dopaminergic system results in increases in prodynorphin-derived peptides in rat basal ganglia and substantia nigra and preprodynorphin mRNA in striatum (11–13). These increases can be produced by treatment with the psychomotor stimulants and indirect-acting agonists methamphetamine and cocaine (11, 13). In contrast to the effects of dopaminergic agonists, chronic antagonist treatment increases preproenkephalin mRNA and proenkephalin-derived peptides (14, 15).

The effects of dopaminergic synaptic transmission are mediated by at least two sets of receptors (for review see ref. 16). D_1 receptors are linked in a positive fashion to adenylate cyclase, whereas D_2 receptors are linked in a negative fashion (16–18). Activation of these receptors also appears to influence transmembrane ionic conductances and even conductance through gap junctions in some tissues (19, 20). Thus, release of dopamine can have effects both on cell firing and on intracellular regulatory mechanisms mediated by cAMP-dependent protein kinases. The coupling of synaptic events to longer-term cellular or behavioral changes (e.g., sensitization in the case of cocaine) has often been hypothesized to involve changes in gene expression. However, the mechanisms and molecules involved in coupling synaptic events with nuclear events have not been fully elaborated. Recently, several classes of nuclear DNA-binding proteins have been identified that bind to specific enhancer elements of genes and thereby modulate transcription (for reviews see refs. 21 and 22). In neurons, the genes coding for several of these proteins are rapidly transcribed and translated in response to a variety of physiological, pharmacological, electrophysiological, and growth-related stimuli (for reviews see refs. 23 and 24). These factors represent proteins transcribed from a set of genes termed cellular immediate-early genes (24). One of the first such proteins to be identified in brain was the c-*fos* protooncogene product, Fos, the cellular homolog of the transforming factor in two feline osteosarcoma retroviruses (25). Because Fos has been suggested to function as an intermediary in regulation of neuropeptide gene expression, including enkephalin (26, 27) and dynorphin (28), we have assessed whether c-*fos* induction is a component of the neuropharmacological actions of cocaine on cells in the rat caudate nucleus.

MATERIALS AND METHODS

Materials. Cocaine hydrochloride was obtained from Sigma; SCH-23390 and sulpiride were from Research Biochemicals (Natick, MA). Immunochemicals were from Accurate Chemicals and Scientific (Westbury, NY) and keyhole limpet hemocyanin and complete Freund's adjuvant were from Calbiochem. The synthetic peptide corresponding to amino acids 128–152 (KVEQLSPEEEEKRRIRRERNKMAAA) of rat Fos (29) was synthesized on an Applied Biosystems peptide synthesizer and was a gift from F. Robey and E. Bonvini (30).

Drug Treatment and Immunocytochemistry. Male Sprague–Dawley rats (250–350 g) were maintained on a 12-hr light/dark cycle and allowed free access to food and water. The rats were injected i.p. with saline vehicle or cocaine (0.3–30 mg/kg of body weight). Antagonists were administered 30 min prior to cocaine. At various times after treatment (1–48 hr) animals were deeply anesthetized with pentobarbital and perfused transcardially with saline followed by 4% paraformaldehyde in 85 mM sodium phosphate (pH 7.4). Brains were

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removed, postfixed for 2 hr, and placed in 30% sucrose in phosphate-buffered saline (PBS) for 2–3 days. Coronal sections (30 μm) were cut on a microtome. Sections from control and treated rats were incubated 60 hr with affinity-purified polyclonal rabbit antiserum diluted 1:20,000 in 1% normal goat serum (NGS) in PBS containing 0.76% Triton X-100. The tissue was washed with PBS and then incubated with goat anti-rabbit IgG diluted 1:200 in 1% NGS/0.76% Triton X-100/PBS for 1 hr. The tissue was washed in PBS and then incubated with rabbit peroxidase–anti-peroxidase complex (diluted 1:5000 with 1% NGS/0.76% Triton X-100/PBS) for 1 hr. Following a final wash, the sections were incubated in 0.05% diaminobenzidine/0.01% H_2O_2 for 6.5 min, washed in PBS, and mounted on gelatin/chrome alum-coated slides. One or more saline-injected control rats were always included with drug-treated animals to control for fixation and tissue processing variables. In general, at the antibody dilution used, control rats displayed few Fos-immunoreactive neurons (see Fig. 2).

Preparation of Antibody. The preparation of the antibody has been described in part (31). Briefly, 20 mg of the synthetic Fos peptide was conjugated to 20 mg of succinylated hemocyanin with 20 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Pierce) in 1.5 ml of H_2O with shaking overnight. The conjugate (20 mg) was dialyzed, lyophilized, dissolved in saline at 500 μg/ml, and emulsified in an equal volume of complete Freund's adjuvant. Antiserum from male New Zealand White rabbits was affinity-purified at 4°C by passing 15 ml of serum over a CNBr-activated Sepharose CH-4B column (Pharmacia) to which peptide (20 mg/1.5 g of column matrix) had been coupled according to the manufacturer's directions. The column was washed with 50 ml of 150 mM NaCl/50 mM Tris·HCl, pH 7.4, and then with the same buffer adjusted to 2 M NaCl until the A_{280} was zero (≈50 ml). Specific antibodies were eluted by incubating the column matrix for 15 min in 10 ml of 4 M $MgCl_2$. Fractions constituting the protein peak were pooled and dialyzed exhaustively against PBS at 4°C.

Isolation of Nuclei. Rats were decapitated and the brains were rapidly removed and placed on an ice-cold glass plate. Striata, ≈40 mg each, from three or four rats were dissected bilaterally, so that the head and part of the tail of the caudate-putamen were represented. Cell nuclei were isolated essentially as described by Tata (32). The striata were homogenized in 3 ml of SMH (0.32 M sucrose/3 mM $MgCl_2$/1 mM Hepes, pH 6.8) in a Potter–Elvehjem homogenizer with a motor-driven Teflon pestle. The crude homogenate was transferred to a centrifuge tube and diluted with 0.6 vol of SMH and 0.22 vol of H_2O. The diluted homogenate was carefully underlaid with 0.8 vol of SMH and centrifuged in a swinging-bucket rotor at 1000 × *g* for 10 min. The supernatant was removed and the pellet was completely resuspended in 5–10 ml of 1.8 M sucrose/1 mM $MgCl_2$/1 mM Hepes, pH 6.8, and spun at 50,000 × *g* in a Beckman SW-55Ti rotor at 4°C. The pellet was recovered in 100–200 μl of 0.25 M sucrose/1 mM $MgCl_2$/1 mM Hepes, pH 6.8. Aliquots (5 or 10 μl) were measured for protein. Purity was checked by brief incubation of a drop of the final nuclear suspension with the DNA-selective fluorescent dye 4′,6-diamino-2-phenylindole (0.1 μg/ml; Fluka) (33) and examined microscopically under oil immersion with bright-field and fluorescence illumination. Nearly every object seen in bright field was also intensely fluorescent and thus contained DNA.

Electrophoresis and Immunoblotting. The nuclear preparations were boiled for 1.5 min in 62.5 mM Tris·HCl, pH 6.8/10% glycerol/2% SDS, 5% 2-mercaptoethanol. A sample equivalent to 30 μg of protein (30–40 μl) was used for analysis. SDS/PAGE (34) was performed using a 4% stacking gel and 10% separating gel at 100 V for 4 hr in a BRL V16 gel apparatus. The gel was then equilibrated for 20 min in transfer buffer (12.5 mM Tris/96 mM glycine/20% methanol, pH 8.3) and proteins were transferred overnight at 20 V to Immobilon P membranes (Millipore), previously wetted in 100% methanol and then water for 2–4 min and placed in transfer buffer.

After transfer, membranes were incubated in blocking solution (120 mM NaCl/1.5% gelatin/0.2% Tween 20/100 mM Hepes, pH 7.4) for 1 hr and then placed in blocking solution containing primary antibody at a dilution of 1:1000 for 1 hr. The membranes were washed twice (10 min each) in blocking solution and then incubated with 0.85 μCi of ^{125}I-labeled protein A (83 μCi/μg; NEN; 1 μCi = 37 kBq) for 1 hr. After two or three washes (10 min each) in 120 mM NaCl/0.2% Tween 20/100 mM Hepes, pH 7.4, the blot was dried between two sheets of filter paper for ≈30 min. Autoradiography was performed for 1–3 days with intensifying screens at −70°C. Reaction in all bands could be blocked by preadsorption of the antibody with the peptide except for the lowest band, migrating with the 14-kDa marker (data not shown). Autoradiograms were scanned with a densitometer and peak areas were determined by weight.

RESULTS

Cocaine treatment resulted in dose-dependent increases in locomotor activity. Stereotypic motor behaviors, including rearing, head-bobbing, and sniffing, were evident at the highest dose. With single injections of the behaviorally active dose of 30 mg/kg, the time course of Fos staining showed a significant increase in immunoreactive neuronal nuclei as early as 1 hr after injection (Fig. 1 *Upper*). Peak increases in numbers of immunoreactive neurons and the intensity of staining occurred at 2 and 4 hr and returned to control levels by 48 hr (Fig. 1 *Upper*). Light counterstaining with cresyl violet confirmed that the Fos staining was neuronal and nuclear. The effect of cocaine on Fos expression was dose-dependent, indicating a graded response (Fig. 1 *Lower*). When assessed 2 hr after treatment, no increase over control in the number of Fos-positive neuronal nuclei was evident in animals receiving 0.3 mg/kg. Administration of 3 mg/kg gave

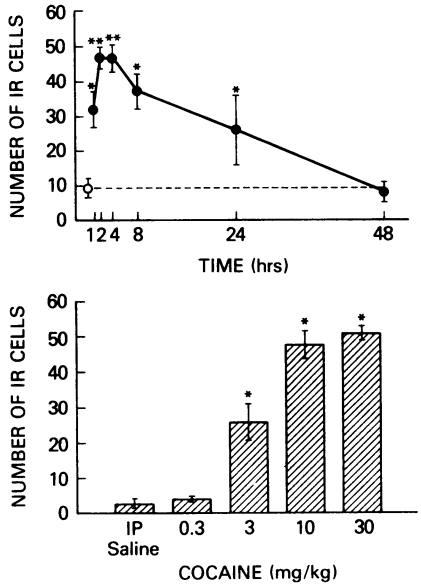

FIG. 1. Time course and dose response of Fos elevation. (*Upper*) Time course of induction of Fos immunoreactivity after cocaine (30 mg/kg, i.p.). The peak increase in immunoreactive (IR) neuronal nuclei occurs between 2 and 4 hr and declines slowly over the ensuing 24 hr. (*Lower*) Dose-dependent increases in Fos immunoreactivity in striatal neuronal nuclei 2 hr after cocaine in rat caudate. Values represent the mean number of stained nuclei counted in five to six 0.33-mm^2 regions from sections in anterior, middle, and posterior caudate of three to six animals per dose. *, $P < 0.05$; **, $P < 0.01$.

an 8-fold increase in the number of striatal cells with Fos immunoreactivity. Even greater numbers of immunoreactive cells were evident in animals treated with cocaine at 10 or 30 mg/kg. The 3-mg/kg dose elicited little behavioral activation, suggesting that the increase in Fos was not a result of cocaine-induced motor activity.

In addition to the number of stained cells, the intensity of staining was notably greater in the cocaine-treated animals, especially those receiving the 30 mg/kg. Fig. 2 *B* and *C* shows a section through the head of the caudate nucleus from a control and a cocaine-treated animal. In these animals, positive striatal neuronal nuclei accumulated so much reaction product that they were nearly black and were fully filled in. In all treatment groups not all neurons stained (Fig. 2), indicating that the effect of cocaine was restricted to certain cells and not generalized to the entire neuronal population of the caudate. Increased numbers of Fos-positive nuclei were counted at all rostrocaudal levels of the caudate; however, the highest density and intensity of immunoreactive neurons were located in the dorsomedial–dorsocentral portions of the anterior caudate. Among other areas that showed increased Fos-positive neuronal nuclei after cocaine treatment were the olfactory tubercle, the islands of Calleja, and the nucleus accumbens; however, these were not subjected to a quantitative analysis as was performed in the caudate.

Western blot characterization demonstrated several Fos-immunoreactive bands. Preliminary studies indicated that optimal detection was obtained with purified cell nuclei. Fig. 3 shows the results obtained from striatal nuclei isolated 2, 4, 24, and 48 hr after a single injection of cocaine compared with saline-treated rats 2 hr after injection. Control nuclei contained barely detectable amounts of the putative 55-kDa Fos protein. Fos has been shown to undergo posttranslational modification and to vary between 55 and 62 kDa (25, 39); in our gel systems it consistently migrated at 55 kDa. In the control lane, the band (actually a doublet) at 41 kDa was abundant whereas comparatively low levels of the bands migrating at 44, 35, and 25 kDa were detected. The 55-kDa Fos protein was detected at 2 and 4 hr after treatment with cocaine (Fig. 3). At 4 hr, increases in the species at 44, 41, 35, and 25 kDa were noted as well. These represent *F*os-*r*elated *a*ntigens (Fra), which are additional proteins from several

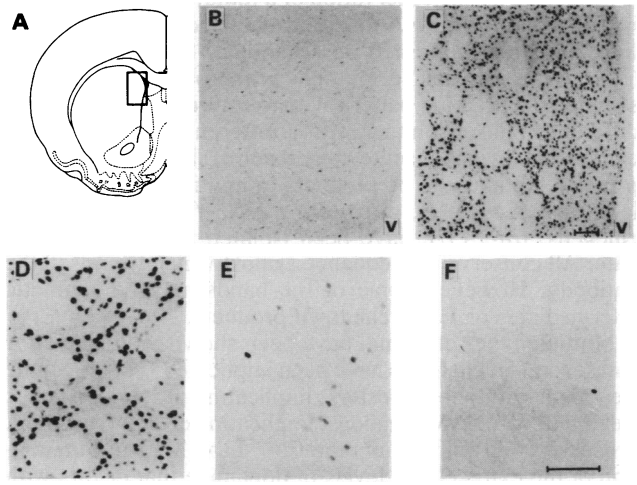

FIG. 2. Fos-immunoreactive neuronal nuclei in rat caudate-putamen following cocaine administration and with pretreatment with the D_1 anatagonist SCH-23390. (*A*) Diagram of forebrain section illustrating the region of the caudate shown in *B–F*. (*B*) Saline-treated control showing low level of basal staining. v, Lateral ventricle. (*C*) Animal injected 2 hr previously with cocaine (30 mg/kg, i.p.) showing widespread induction of Fos. (Bar = 100 μm.) (*D–F*) High-power view of caudate from rats treated with cocaine (30 mg/kg) for 2 hr without pretreatment (*D*) or pretreated with SCH-23390 at 10 μg/kg (*E*) or 50 μg/kg (*F*). With the higher dose of SCH-23390 the staining was nearly completely suppressed; the object in the field is a red blood cell remaining from the perfusion. (Bar = 100 μm.) Preadsorption of the antiserum with antigenic peptide (1 μM) abolished all staining (data not shown).

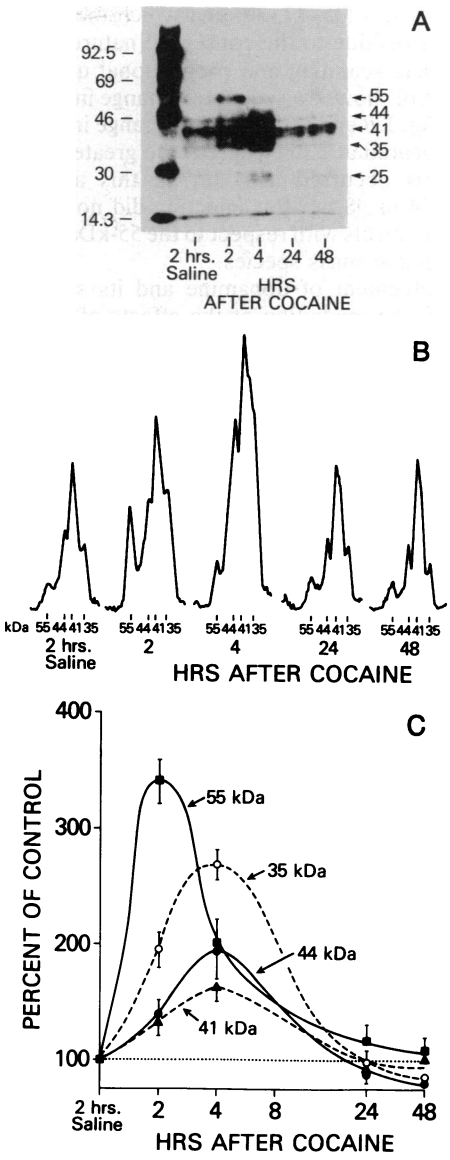

FIG. 3. Western blot analysis of Fos and Fos-related antigens. (*A*) Autoradiograms of Fos-immunoreactive proteins in striatal nuclei isolated by centrifugation (32) 2, 4, 24, and 48 hr after injection of cocaine (30 mg/kg, i.p.) Numbers at left correspond to the molecular size markers (partially obscured by a blotch on the film). Numbers at right (arrows) correspond to the molecular masses (kDa) of Fos-immunoreactive proteins distinguishable in the autoradiogram. The band at 55 kDa represents the putative Fos protein. Bands migrating between 44 and 25 kDa have been termed Fos-related antigens (35–37) and are referred to collectively as the Fra complex. (*B*) Densitometric scan (the trace proceeds from the top to the bottom of each lane) shows a rapid elevation in the 55-kDa Fos band at 2 hr and a delayed elevation in the Fra complex at 4 hr. (*C*) Proportional changes in the Fos-immunoreactive proteins. The mean peak heights from four scans of several exposures were determined and plotted as a percentage of the 2-hr saline control. A similar set of data was obtained when the gel was scanned horizontally (across each band) and integrated according to peak area. Multiple Fos-immunoreactive bands have also been observed in Western blots from spinal cord during peripheral inflammation by using the same antibody (38) and in hippocampus by using a similar antibody (to the same epitope) after seizures (39).

fos-related genes (35–37, 39) and which the primary antiserum recognizes due to the conserved nature of the epitope. Densitometric scanning and proportional quantitation relative to control showed a sequential change in the Fos and Fra proteins (Fig. 1 *B* and *C*). The peak change in the 55-kDa *Fos* protein appeared at 2 hr, whereas the greatest change in the Fra proteins occurred at 4 hr. In this analysis animals sacrificed 24 or 48 hr after injection did not differ substantially from controls with respect to the 55-kDa protein and the lower molecular mass species.

The involvement of dopamine and its specific receptor subtypes in the mediation of the effects of cocaine on Fos expression was investigated by the administration, 30 min prior to cocaine (30 mg/kg) treatment, of the selective D_1 and D_2 receptor antagonists, SCH-23390 (0.01 and 0.05 mg/kg, i.p.) and sulpiride (30, 50, and 100 mg/kg, i.p.), respectively (see ref. 16). Both antagonists produced partial, but not complete, suppression of cocaine-elicited locomotor activity and stereotypic behaviors at the doses tested. SCH-23390 at 0.01 mg/kg produced a partial block of the effects of cocaine on Fos expression as assessed immunohistochemically (Figs. 2 *D–F* and 4 *Upper*). The higher dose, 0.05 mg/kg, completely blocked the effect of cocaine on Fos expression in all animals, with the number of stained nuclei at or below control levels. These data suggest a significant role for D_1 receptors in mediating induction of Fos by cocaine. Administration of sulpiride yielded variable results with Fos expression unchanged at 30 mg/kg but partially blocked by the 50 mg/kg and 100 mg/kg doses (Fig. 4 *Lower*). It is possible that the partial block by the two higher doses of sulpiride was due to loss of receptor selectivity (i.e., via an action on D_1 receptors). These data do not rule out a role for D_2 receptors in mediating cocaine's action on Fos expression but suggest, at the very least, that the role of D_1 receptors is more salient than that of D_2.

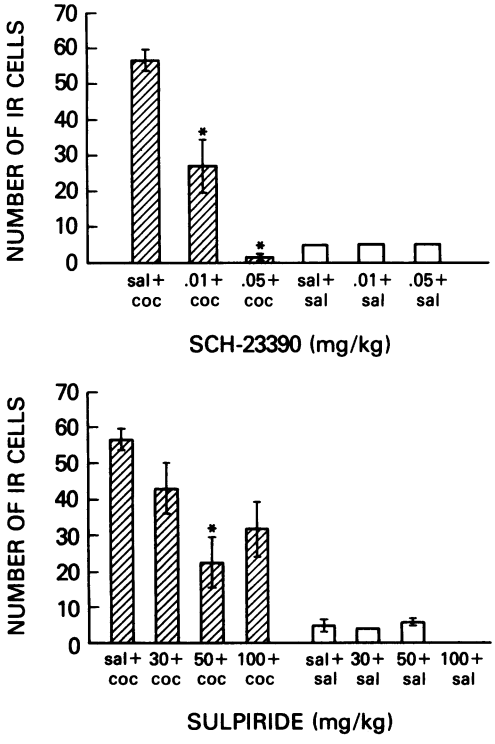

FIG. 4. Effect of selective D_1 and D_2 antagonists on induction of Fos-immunoreactive (IR) neurons by cocaine in rat caudate. Saline (sal), SCH-23390 (0.01 or 0.05 mg/kg), or sulpiride (30, 50, or 100 mg/kg) was administered i.p. 0.5 hr prior to cocaine (coc; 30 mg/kg, i.p.) or saline; animals were sacrificed 2 hr after treatment with cocaine. (*Upper*) Effective, dose-related block of cocaine-induced increase in Fos immunoreactivity by SCH-23390, a selective D_1 antagonist. (*Lower*) Relatively ineffective block of cocaine's effect by sulpiride, a selective D_2 antagonist. Neither antagonist given alone had an effect on Fos-like immunoreactivity. *, $P < 0.05$.

DISCUSSION

Our observations that Fos and Fra proteins are induced by cocaine administration demonstrate an effect of cocaine at the level of gene expression and, potentially, upon gene regulation. The induction is dose-dependent and has an extended time course, with effects noted immunocytochemically out to 24 hr after treatment. Not every striatal neuron is affected, even at the relatively high 30-mg/kg dose. This suggests that only a specific subpopulation of striatal neurons is affected. The effects of receptor-selective dopamine antagonists indicate that the D_1 subtype predominates in mediating the increase in Fos expression. Western blot analysis indicates that the tissue immunoreactivity in this brain region is composed of multiple protein species. These Fos-immunoreactive proteins show a sequential up-regulation following cocaine administration, suggesting an important temporal component to Fos-related processes.

The multiple Fos proteins seen in the Western blot are a feature common to several regions of the central nervous system when appropriate stimuli for Fos induction are administered. In spinal cord we have also observed bands at 55, 44, 41, and 38 kDa (but not at 35 kDa) during a peripheral inflammatory stimulus (38). The Fos proteins recognized by our antibody will bind to the AP-1 (40) consensus sequence, TGA(G) or (C)TCA: the antibody produces a specific gel retardation pattern when added to AP-1 complexes formed with the gibbon leukemia virus enhancer and extracts of HeLa cells (31). Both the 55-kDa Fos and the Fra proteins can be detected by Western blot of nuclear extracts after affinity purification over a multimeric AP-1 affinity column (31). Thus, by several criteria this antibody recognizes Fos proteins and these proteins are capable of constituting an AP-1 complex. Further, the basic phenomenon of c-*fos* induction is supported by the observation of a rapid, transient, marked increase in c-*fos* mRNA following acute cocaine administration (M.J.I., unpublished observations). Sonnenberg *et al.* (39) have also detected multiple Fos proteins as well as a sequential activation pattern with Western blots, albeit in a different brain region and with a different stimulus (hippocampus following an acute convulsion). These neuronal Fos proteins are likely to be products of multiple genes since at least four *fos* genes, c-*fos*, *fra-1* (35), *fos-B* (36), and in chickens, *fra-2* (37), have been isolated and sequenced to date. All conserve the sequence against which we raised the antibody. However, some of the bands may be synthetic intermediates or Fos breakdown products.

Multiple types of stimuli have been shown to induce c-*fos* and several mechanisms have been implicated. These include treatment with phorbol esters, implicating protein kinase C; treatment with cAMP analogs, implicating cAMP-dependent processes; and transmembrane Ca^{2+} flux (23, 24). Potentiation of the extracellular levels of dopamine that occurs with cocaine (41) might be expected to enhance cAMP production via activation of D_1 receptors (16, 17). Since the c-*fos* promoter contains one or more cAMP response elements (CREs) (42–45), we hypothesize that stimulation of dopamine-dependent adenylate cyclase, and a subsequent action at one of the c-*fos* CREs, is a likely mechanism for increased c-*fos* expression. Positive regulation of c-*fos* expression by adenylate cyclase stimulation has been demonstrated in several cultured neuronal and non-neuronal systems (e.g., 46, 47). In making this proposal for dopamine in striatum, the apparent paradox of simultaneous activation of D_2 receptors linked in a negative fashion to adenylate cyclase must be considered. Several factors suggest a more complex situation

than a simple opposition of the two receptors. (*i*) It has not been unequivocally established that these two receptors are on the same cell. We have observed that the Fos response occurs on many but not all striatal neurons; thus, there is heterogeneity at the cellular level in striatum. (*ii*) Extracellular electrophysiological recordings have shown that simultaneous activation of D_1 and D_2 receptors can synergize to generate an increased firing rate in neurons receiving dopaminergic innervation (19). (*iii*) Diversity at the level of the D_2 receptor protein has been demonstrated to exist via alternative RNA splicing (48–51). The differential splicing occurs in the cytoplasmic loop that interacts with guanine nucleotide-binding proteins. It is possible that D_1–D_2 receptor interactions may be modified by which spliced D_2 isoform is expressed in a particular neuron, thereby providing heterogeneity at the biochemical level.

Regardless of potential D_1–D_2 receptor interactions, use of antagonists or agonists indicates a proximal role for D_1 receptors in mediating the increase in Fos proteins. We obtained effective blockade of the Fos increase by pretreatment with SCH-23390, a selective D_1 antagonist, and no clearly dose-related blockade with sulpiride, a D_2 antagonist. The blockade with SCH-23390 at 50 μg/kg was as complete as preadsorption control experiments with the antigen peptide. The involvement of D_1 receptors is supported by a selective increase in Fos immunoreactivity after administration of the D_1-specific agonists SKF-38393 and Cy208-243 and not by a D_2-specific agonist (52). One of the interesting observations to be gleaned from the present study and that with the agonists (52) is that in the intact striatum, indirect agonists appear to be more effective in inducing c-*fos* than receptor-selective direct agonists. The reasons for this, as discussed above, are not clear but may be consistent with the idea that activation of a repertoire of receptor types (e.g., by use of re-uptake blockers) is the most effective pathway for Fos induction.

Fos is a known trans-acting protein and is hypothesized to be involved in controlling the expression of a variety of neuronal genes. Hippocampal seizure activity initially elevates c-*fos* and then enkephalin gene expression (26, 27, 39); a similar situation occurs for c-*fos* and the dynorphin gene in rat spinal cord in response to noxious stimulation (28). In striatum, dynorphin gene expression is also increased by chronic administration of the dopaminergic agonists methamphetamine and cocaine (11–13). In fact it was observations in the spinal cord (28) that suggested the striatal dynorphin increase might be preceded by an increase in Fos. The increase in Fos, the Fra proteins, and, it is likely, several other classes of nuclear protooncogene products might serve as the initial triggers and regulatory factors for longer-term changes in a network of striatal genes. Our data demonstrate that cocaine produced increased numbers of Fos-immunoreactive neurons up to 24 hr after a single treatment, long after cocaine had been cleared from the system. Thus, even a single dose of cocaine may produce long-lasting effects on neuronal biochemistry by transcriptional modulation.

Further analysis of cellular immediate-early gene activation may provide insight into some of the long-term behavioral consequences of cocaine usage. The involvement of Fos in the biochemical mechanisms of dopamine also provides an avenue for the investigation of neuropsychiatric disorders that involve the brain dopaminergic systems, such as schizophrenia and Parkinson disease.

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1. Heikkila, R. E., Orlansky, H. & Cohen, G. (1975) *Biochem. Pharmacol.* **24**, 847–852.
2. Moore, K. E., Chiueh, C. C. & Zeldes, G. (1977) in *Cocaine and Other Stimulants*, eds. Ellinwood, E. H. & Kilbey, M. M. (Plenum, New York), pp. 143–160.
3. Koe, K. B. (1976) *J. Pharmacol. Exp. Ther.* **199**, 649–661.
4. Ross, S. F. & Renyi, A. L. (1967) *Life Sci.* **6**, 1407–1415.
5. Hertting, G., Axelrod, J. & Whitby, L. G. (1961) *J. Pharmacol. Exp. Ther.* **134**, 146–153.
6. Goeders, N. E. & Smith, J. E. (1983) *Science* **221**, 773–775.
7. DeWit, H. & Wise, R. A. (1977) *Can. J. Psychol.* **31**, 195–203.
8. Risner, M. E. & Jones, B. E. (1980) *Psychopharmacology* **71**, 83–93.
9. Fallon, J. & Moore, R. J. (1978) *J. Comp. Neurol.* **180**, 545–580.
10. Palkovits, M., Brownstein, M. J. & Zamir, N. (1984) *Neuropeptides*, **4**, 193–199.
11. Sivam, S. P. (1989) *J. Pharmacol Exp. Ther.* **250**, 818–824.
12. Li, S. J., Sivam, S. P., McGinty, J. F., Jiang, H. K., Douglass, J., Calavetta, L. & Hong, J. S. (1988) *J. Pharmacol. Exp. Ther.* **246**, 403–408.
13. Hanson, G. R., Merchant, K. M., Letter, A. A., Bush, L. & Gibb, J. W. (1987) *Eur. J. Pharmacol.* **144**, 245–246.
14. Hong, J. S., Yoshikawa, K., Kanamatsu, T. & Sabol, S. C. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 2535–2539.
15. Tang, F., Costa, E. & Schwartz, J. P. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3841–3844.
16. Clark, D. & White, F. J. (1987) *Synapse* **1**, 347–388.
17. Stoof, J. C. & Kebabian, J. W. (1981) *Nature (London)* **294**, 366–368.
18. Cooper, D. M. F., Bier-Laning, C. M., Halford, M. K., Ahlijanian, M. K. & Zahniser, N. R. (1986) *Mol. Pharmacol.* **29**, 113–119.
19. Walters, J., Bergstrom, D. A., Carlson, J. H., Chase, T. N. & Braun, A. R. (1987) *Science* **236**, 719–722.
20. Rogawski, M. (1987) *Trends Neurosci.* **10**, 200–205.
21. Mitchell, P. M. & Tjian, R. (1989) *Science* **245**, 371–378.
22. Marriott, S. J. & Brady, J. N. (1989) *Biochim. Biophys. Acta* **989**, 97–110.
23. Morgan, J. I. & Curran, T. (1989) *Trends Neurosci.* **12**, 459–462.
24. Sheng, M. & Greenberg, M. E. (1990) *Neuron* **4**, 477–485.
25. Curran, T., Miller, A. D., Sokas, L. & Verma, I. (1984) *Cell* **36**, 259–268.
26. White, J. D. & Gall, C. (1987) *Mol. Brain Res.* **3**, 21–29.
27. Sonnenberg, J. L., Rauscher, F. J., III, Morgan, J. I. & Curran, T. (1989) *Science* **246**, 1622–1625.
28. Draisci, G. & Iadarola, M. J. (1989) *Mol. Brain Res.* **6**, 31–37.
29. Curran, T., Gordon, M. B., Rubino, K. L. & Sambucetti, L. C. (1987) *Oncogene* **2**, 79–84.
30. Giardina, S. L., Evans, S. W., Gandino, L., Robey, F. A., Bonvini, E., Longo, D. L. & Varesio, L. (1987) *Anal. Biochem.* **161**, 109–116.
31. Quinn, J. P., Takimoto, M., Iadarola, M., Holbrook, N. & Levens, D. (1989) *J. Virol.* **63**, 1737–1742.
32. Tata, J. R. (1974) *Methods Enzymol.* **31A**, 253–257.
33. Russell, W. C., Newman, C. & Williamson, D. H. (1975) *Nature (London)* **253**, 461–462.
34. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
35. Cohen, D. R. & Curran, T. (1988) *Mol. Cell. Biol.* **8**, 2063–2069.
36. Zerial, M., Toschi, L., Ryseck, R.-P., Schuermann, M., Muller, R. & Bravo, R. (1989) *EMBO J.* **8**, 805–813.
37. Nishina, H., Sato, H., Suzuki, T., Sato, T. & Iba, H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3619–3623.
38. Iadarola, M. J., Yeung, C. L., Draisci, G. & Ruda, M. A. (1989) *Soc. Neurosci. Abstr.* **15**, 468.
39. Sonnenberg, J. L., Macgregor-Leon, P. F., Curran, T. & Morgan, J. I. (1989) *Neuron* **3**, 359–365.
40. Curran, T. & Franza, B. R., Jr. (1988) *Cell* **55**, 395–397.
41. Di Chiara, G. & Imperato, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5274–5278.
42. Berkowitz, L. A., Riabowol, K. L. & Gilman, M. Z. (1989) *Mol. Cell. Biol.* **9**, 4272–4281.
43. Hyman, S. E., Comb, M., Lin, Y.-S., Pearlberg, J., Green, M. R. & Goodman, H. M. (1988) *Mol. Cell. Biol.* **8**, 4225–4233.
44. Sassoni-Corsi, P., Visvader, J., Ferland, L., Mellon, P. L. & Verma, I. (1988) *Genes Dev.* **2**, 1529–1538.
45. Bravo, R., Neuberg, M., Burckhardt, J., Almendral, J., Wallich, R. & Muller, R. (1987) *Cell* **48**, 251–260.
46. Kousvelari, E. E., Louis, J. M., Huang, L.-H. & Curran, T. (1988) *Exp. Cell Res.* **179**, 194–203.
47. Mochetti, I., De Bernardi, M. A., Szekely, A. M., Alho, H. M., Brooker, G. & Costa, E. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3891–3895.
48. Bunzow, J. R., Van Tol, H. H. M., Grandy, D. K., Albert, P., Salon, J., Christie, M., Machida, C. A., Neve, K. A. & Civelli, O. (1988) *Nature (London)* **336**, 783–787.
49. Giros, B., Sololoff, P., Martres, M.-P., Riou, J.-F., Emorine, L. J. & Schwartz, J.-C. (1989) *Nature (London)* **342**, 923–926.
50. Monsma, F. J., Jr., McVittie, L. D., Gerfen, C. R., Mahan, L. H. & Sibley, D. R. (1989) *Nature (London)* **342**, 926–929.
51. Chio, C. L., Hess, G. F., Graham, R. S. & Huff, R. M. (1990) *Nature (London)* **343**, 266–269.
52. Robertson, H. A., Peterson, M. R., Murphy, K. & Robertson, G. S. (1989) *Brain Res.* **503**, 346–349.