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DARPP-32, A DOPAMINE- AND ADENOSINE 3′:5′-MONOPHOSPHATE-REGULATED PHOSPHOPROTEIN ENRICHED IN DOPAMINE-INNERVATED BRAIN REGIONS

I. Regional and Cellular Distribution in the Rat Brain[1]

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Abstract

The present study documents the existence in mammalian brain of a phosphoprotein which may play a biological role in dopaminoceptive neurons. This protein has been designated DARPP-32 (*d*opamine- and *a*denosine 3′:5′-monophosphate-*r*egulated *p*hospho*p*rotein-*32*,000). The regional distribution of DARPP-32 in the rat brain follows the general pattern of dopaminergic innervation. DARPP-32 is present in dopaminoceptive rather than dopaminergic neurons. Moreover, it appears to be concentrated in a subpopulation of dopaminoceptive cells, namely those containing D-1 receptors (dopamine receptors coupled to adenylate cyclase activation), where it is localized in cell bodies, dendrites, axons, and nerve terminals. DARPP-32 is phosphorylated in intact cells from the caudatoputamen by dopamine and by 8-bromo-cyclic adenosine 3′:5′-monophosphate and in cell-free preparations by cyclic adenosine 3′:5′-monophosphate-dependent protein kinase.

In two accompanying papers, we report the purification and biochemical characterization of DARPP-32 (Hemmings, H. C., Jr., A. C. Nairn, D. W. Aswad, and P. Greengard (1984) J. Neurosci. *4:* 99–110) and the immunocytochemical localization of this phosphoprotein (Ouimet, C. C., P. Miller, H. C. Hemmings, Jr., S. I. Walaas, and P. Greengard (1984) J. Neurosci. *4:* 111–124).

The presence of a large number of neuron-specific phosphoproteins has recently been demonstrated in the mammalian CNS (Walaas et al., 1983a, b). Some of these phosphoproteins displayed highly restricted regional distributions, suggesting a possible relationship to specific neurotransmitter systems. One of these phosphoproteins, with apparent M_r = 32,000, was phosphorylated by a cAMP-dependent protein kinase and appeared to be enriched in the basal ganglia. We have now undertaken a detailed study of this protein, designated DARPP-32 (*d*opamine- and *a*denosine 3′:5′-monophosphate-*r*egulated *p*hospho*p*rotein-*32*,000). The present paper reports the results of studies on the regional distribution of DARPP-32 in the brain, on its cellular and subcellular localization, and on the phosphorylation of the protein, in selected parts of the basal ganglia. The two accompanying papers report the results of the purification and characterization of DARPP-32 (Hemmings et al., 1984) and the results of an immunocytochemical analysis of the brain regions and cells which contain DARPP-32 (Ouimet et al., 1984). A brief description of some of the results described in these three papers has been given elsewhere (Walaas et al., 1983c).

Materials and Methods

Materials. [γ-^{32}P]ATP was synthesized by the method of Glynn and Chappell (1964). Other radiochemicals were from New England Nuclear. *Staphylococcus aureus* V8 protease was from Miles Biochemicals. Trasylol was from Mobay Chemical Corp. Pepstatin A, phenylmethylsulfonyl fluoride, kainic acid, dopamine (3-hydroxytyramine), serotonin (5-hydroxytryptamine creatinine sulfate), L-norepinephrine, 6-hydroxydopamine, and 8-bromo-cAMP were from Sigma Chemical Co. Isobutyl-

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methylxanthine (IBMX) was from Aldrich. Fluphenazine hydrochloride was from Squibb. Phosphatidylserine (Sigma) was suspended in 20 mM Tris-HCl (pH 7.5) as described (Walaas et al., 1983a). Calmodulin and the catalytic subunit of cAMP-dependent protein kinase, both highly purified, were kindly provided by Dr. Angus C. Nairn of this laboratory. Other chemicals were obtained from standard commercial suppliers.

Animals. Male Sprague-Dawley rats (150 to 200 gm body weight) were used in all experiments. They were stunned and decapitated, and their brains were rapidly placed in ice-cold 0.32 M sucrose/1 mM HEPES (pH 7.4). In some experiments the spinal cord, the anterior and posterior pituitaries, and samples of the kidney, spleen, lung, heart, liver, and skeletal muscle were also dissected.

Preparation of lesioned animals. Rats were anesthetized with chloral hydrate (350 mg/kg, i.p.) and mounted on a stereotactic frame. In one group of animals, a transverse slot was drilled on the left side of the skull 1 to 2 mm behind the bregma, and the left hemisphere was transected at the level of the globus pallidus with a rectangular knife blade lowered to the base of the skull (Fig. 1). This lesion extended from the midline to the deep layers of the lateral cortex and severed all relevant subcortical structures. A second group of animals was subjected to intracerebral infusions of either kainic acid (5 nmol in 1 μl of phosphate-buffered saline) or 6-hydroxydopamine (6 μg in 4 μl of Krebs-Ringer buffer (KRB) containing 1 mM ascorbic acid). Some animals received infusions into the rostral caudatoputamen at coordinates A:8000, L:2500, V:+700 as described (Walaas, 1981), whereas other animals received infusions into the substantia nigra at coordinates A:2600, L:1500, DV:−2500 (König and Klippel, 1963). The needle was left in place after these infusions for 3 min to prevent backflow. All lesioned animals were allowed to survive for 5 to 7 days to ensure anterograde degeneration of lesioned nerve fibers (Storm-Mathisen, 1975), and then processed together with untreated controls for quantitative analysis of phosphoproteins and neurotransmitter markers.

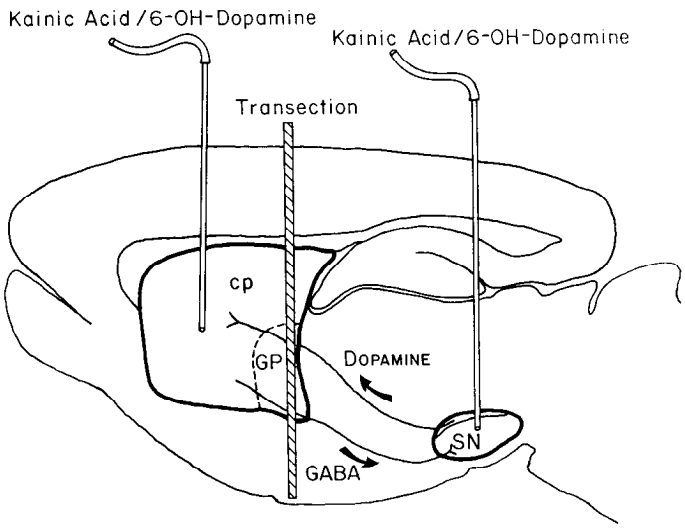

Figure 1. Schematic drawing based on a parasagittal section of rat brain, showing the site of hemitransection (*bar*) and the placement of cannulae for infusion of neurotoxins. *Arrows* indicate ascending dopaminergic nigrostriatal and descending GABAergic striatonigral pathways. *SN*, substantia nigra; *cp*, caudatoputamen; *GP*, globus pallidus.

Preparation of brain regions. Some brains were mounted on a McIlwain tissue chopper, and 0.7- to 1-mm frontal slices were prepared through the forebrain and mesencephalon. These slices allowed dissection of the frontal cortex (which included all cortex rostral to the corpus callosum), the caudatoputamen, nucleus accumbens, olfactory tubercle, septum, globus pallidus, amygdala, hypothalamus, thalamus, and substantia nigra as described (Walaas et al., 1983a), following the atlas of König and Klippel (1963). Other brains were used for dissection of the whole neocortex, the hippocampus, the olfactory bulb, pons/medulla, the inferior and superior colliculi, and cerebellum, as described (Walaas et al., 1983a).

The samples from various brain regions were weighed and added to 10 vol of ice-cold "standard buffer" containing 10 mM Tris-HCl (pH 7.4), 2 mM EDTA, 1 mM dithiothreitol, and the protease inhibitors phenylmethylsulfonyl fluoride (0.1 mM), pepstatin A (2 μg/ml) and Trasylol (50 units/ml). The samples were homogenized with 10 strokes in a glass-Teflon homogenizer rotating at 2100 rpm. All samples were then kept on ice while aliquots were subjected to protein determination, which was done with a detergent-based Folin-reagent assay using bovine serum albumin as standard (Peterson, 1977).

Preparation of subcellular fractions. Subcellular fractions from caudatoputamen and substantia nigra were prepared by differential centrifugation (Whittaker and Barker, 1972). Ten caudatoputamens were dissected, pooled, and homogenized in 10 vol of 0.32 M sucrose/1 mM HEPES (pH 7.4) by 10 strokes in a glass-Teflon homogenizer rotating at 900 rpm. The homogenate was centrifuged at 900 × *g* for 10 min and the pellet was washed once in the original volume of sucrose/HEPES to yield a crude nuclear fraction (P_1). The pooled supernatants were centrifuged at 12,000 × *g* for 20 min and the pellet was washed once to yield a crude synaptosomal pellet (P_2), which also contained mitochondria. The supernatants were pooled and centrifuged at 150,000 × *g* for 30 min to produce a microsomal pellet (P_3) and a cytosol fraction (S).

For fractionation of substantia nigra, 10 substantiae nigrae were pooled and homogenized as described above in 20 vol of 0.32 M sucrose/1 mM HEPES (pH 7.4). The homogenate was centrifuged at 17,000 × *g* for 30 min, and the pellet was washed once in the original volume of sucrose/HEPES and recentrifuged to yield a particulate fraction (P). The supernatants were pooled to constitute a soluble fraction (S).

All particulate fractions were resuspended by means of glass-Teflon homogenizers in the original volume of standard buffer. Samples were then kept on ice and analyzed for phosphoproteins, protein concentration, and subcellular marker enzymes.

Preparation of acid extracts. Homogenate samples in standard buffer or subcellular fractions (0.1 to 0.5 ml)

were incubated in polypropylene tubes at 30°C for 20 min to allow dephosphorylation of phosphoproteins by endogenous protein phosphatases. Separate experiments showed that more than 90% of ^{32}P-labeled purified DARPP-32 (Hemmings et al., 1984) added to caudatoputamen homogenates became dephosphorylated during this incubation, and similar results have been found for the synaptic vesicle-associated phosphoprotein synapsin I (Sieghart et al., 1979). Five milliliters of cold 5 mM zinc acetate were then added, and precipitated proteins were collected by centrifugation at 4,000 × *g* for 15 min. The pellets were resuspended in cold 10 mM citrate-phosphate buffer (pH 2.8 to pH 3.0) containing 0.1% (v/v) Triton X-100 and 2 μg/ml of pepstatin A by vigorous vortexing twice for 30 sec. After 10 min on ice, acid-insoluble proteins were removed by centrifugation at 27,000 × *g* for 15 min. The supernatants were neutralized (final pH 6.0 to 6.5) by transfer of aliquots to 1/10 vol of 0.5 M Na_2HPO_4, and centrifuged at 27,000 × *g* for 15 min. The final extracts were kept on ice while protein concentration was determined, using either a Folin-reagent assay (Peterson, 1977) or a dye-binding assay (Bradford, 1976) which is unaffected by monoamines. The two methods gave similar results. Following protein determination, all extracts were adjusted to the same protein concentration. In initial experiments, more than 90% of ^{32}P-labeled DARPP-32 (Hemmings et al., 1984) added to brain homogenates was found to be recovered in the final neutralized extract after this extraction procedure (data not shown). This procedure is also able to extract certain other nervous tissue-specific phosphoproteins, such as synapsin I (Ueda and Greengard, 1977) and proteins IIIa and IIIb (Huang et al., 1982), with high efficiency, while it inactivates endogenous protein kinases and protein phosphatases (Forn and Greengard, 1978).

Phosphorylation of proteins in acid extracts. The phosphoproteins present in equal aliquots of the acid extracts were quantitated by "back-phosphorylation" in a medium (final volume 0.1 ml) containing (final concentration) 50 mM HEPES (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 25 nM of the catalytic subunit of cAMP-dependent protein kinase, and 10 μM [γ-^{32}P]ATP (10 to 30 Ci/mmol). After addition of SDS-containing stop solution (Ueda and Greengard, 1977), the phosphoproteins were separated by SDS-PAGE (Laemmli and Favre, 1973). The gels were fixed, stained, destained, and dried, and the phosphoproteins were visualized by autoradiography (Ueda and Greengard, 1977). ^{32}P incorporation into specific protein bands was quantitated by excision of the bands from the dried gels using the autoradiograms as guides and liquid scintillation spectrometry of the gel pieces.

^{32}P incorporation into DARPP-32 and other phosphoproteins under these conditions was maximal after 45 min incubation at 30°C and remained stable for at least 2 hr of continued incubation. Since the proteins were dephosphorylated by endogenous phosphatases prior to acid extraction, this maximal phosphorylation gives a measure of the total amount of phosphoprotein present. ^{32}P incorporation into DARPP-32 was proportional to total protein content in the incubation mixture up to at least 70 μg (not shown). The standard phosphorylation reactions were therefore performed with 5 to 50 μg of extracted protein, incubated at 30°C for 60 min. The incubation conditions used in the phosphorylation assay resulted, in the case of synapsin I, in ^{32}P incorporation into tryptic phosphopeptide-1, which contains the phosphorylation site specific for cAMP-dependent protein kinase (Huttner et al., 1981), but not into other phosphorylation sites in the synapsin I molecule. These results indicate that the standard phosphorylation conditions caused phosphorylation of substrates for cyclic AMP-dependent protein kinase but not of substrates for other protein kinases.

Demonstration of phosphoproteins in caudatoputamen cytosol. Crude cytosol preparations from caudatoputamen samples were prepared as described (Walaas et al., 1983a, b). Briefly, 1 to 2 ml of the homogenate prepared in standard buffer were centrifuged at 150,000 × *g* for 30 min at 4°C and the supernatants were collected on ice. The pellets were resuspended by vortexing in the same volume of standard buffer and were subjected to a second centrifugation. The two supernatants were pooled to constitute a total soluble fraction, which was dialyzed against standard buffer as described (Walaas et al., 1983b). Aliquots containing 20 to 50 μg of protein were phosphorylated in a medium (final volume, 0.1 ml) containing (final concentration) 25 mM Tris-HCl (pH 7.4), 6 mM $MgSO_4$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, and 2 μM [γ-^{32}P]ATP (10 to 30 Ci/mmol). Endogenous cAMP-dependent or calcium-dependent protein kinases were selectively activated by inclusion of either 2 μM cAMP plus 1 mM IBMX, or of 1.5 mM $CaCl_2$ in the absence or presence of 10 μg/ml of calmodulin or 50 μg/ml of phosphatidylserine, as described (Walaas et al., 1983a, b). The samples were preincubated for 90 sec at 30°C and incubated with [γ-^{32}P]ATP for 60 sec, and the reaction was terminated by SDS-stop solution and boiling for 2 min. The phosphoproteins were then separated by SDS-PAGE and visualized by autoradiography, as described above.

Analysis of the state of phosphorylation of DARPP-32 and of synapsin I in caudatoputamen slices. For preparation of slices, brains were put in cold sucrose as described above and allowed to cool for 1 to 2 min, and the caudatoputamen was then dissected by opening the lateral ventricles and "scooping out" the nucleus with a spatula. For each hemisphere, the caudalmost part of the nucleus was removed, the remaining tissue was placed on a McIlwain tissue chopper and cut into 0.4-mm frontal slices, and the preparation was rapidly put into a glass homogenizer containing 10 ml of oxygenated KRB (pH 7.4 at 30°C, containing in mM: Na^+, 150; K^+, 5; Ca^{2+}, 1.0; Mg^{2+}, 1.5; PO_4^{-3}, 1.5; HCO_3^-, 25; Cl^-, 128.5; ascorbate, 1; D-glucose, 10). The medium was gassed continuously with H_2O-saturated 95% O_2, 5% CO_2 throughout the preincubation and incubation periods. Time between decapitation of the animals and start of the preincubation was 5 to 7 min. To avoid variability between animals, slices from one caudatoputamen were always compared to slices from the contralateral sample, the right or left caudatoputamen being randomly used as experimental or control preparation.

Slices were preincubated for 65 min with a change of

KRB after 30 min. In one series of experiments, where 8-bromo-cAMP, IBMX, or high KCl concentrations were tested, KRB was changed again after 60 min, and the test substances, dissolved in KRB, were added after a further 5 min of preincubation. In another series of experiments, where neurotransmitters were tested, IBMX (final concentration, 20 μM) was added together with the fresh KRB after 60 min of preincubation. (This concentration of IBMX did not by itself induce a significant change in the state of phosphorylation of DARPP-32 or synapsin I in the caudatoputamen slices (not shown).) The test substances were then added to the medium after a further 5 min of preincubation. The final incubation volume was 10 ml.

To determine the amounts of dephospho-DARPP-32 and of dephospho-synapsin I, the incubations were terminated by aspirating the KRB and rapidly homogenizing the slices in 5 ml of cold zinc acetate (5 mM), a procedure which arrests phosphatase activity (Forn and Greengard, 1978). The amounts of the dephospho forms of DARPP-32 and of synapsin I were quantitated by acid-extracting the proteins and phosphorylating them as described above.

To determine the total amounts of DARPP-32 and of synapsin I, slices were homogenized in 0.5 ml of ice-cold buffer containing 10 mM Tris-HCl (pH 7.4), 2 mM EDTA, and the protease inhibitor Trasylol (50 units/ml). The Tris-buffered samples were then incubated at 30°C for 20 min to allow complete dephosphorylation to take place. The reaction was terminated by adding 5 ml of cold zinc acetate (5 mM). The total amounts of DARPP-32 and of synapsin I were quantitated by acid-extracting the proteins and phosphorylating them as described above.

In some experiments there was a significant extent of proteolysis of DARPP-32 but not of synapsin I during the preincubation period, as measured by phosphorylation assay (and confirmed by radioimmunoassay). Data obtained in such experiments (about one-fifth of all experiments) were not included in the final calculations. Statistical significance of differences between the remaining control and treated slices was calculated by the nonparametric Wilcoxon paired sample test (Wilcoxon, 1945).

Miscellaneous methods. Peptide mapping after limited proteolysis with *S. aureus* V8 protease was performed by the method of Cleveland et al. (1977) as described by Huttner and Greengard (1979). Previously developed methods were used for analysis of the neurotransmitter enzymes choline acetyltransferase (ChAT) (Fonnum, 1969), tyrosine hydroxylase (Hendry and Iversen, 1971), and glutamate decarboxylase (GAD) (Maderdrut, 1979). The mitochondrial marker fumarase (Racker, 1950) and the cytosol marker lactate dehydrogenase (Johnson, 1960) were analyzed as described by Whittaker and Barker (1972).

Results

Regional distribution of DARPP-32. Figure 2 shows an autoradiogram of phosphoproteins, present in acid extracts from 18 parts of the rat CNS, and from the anterior and posterior pituitary gland, which were phosphorylated by cAMP-dependent protein kinase. DARPP-32 was found to have an extremely uneven distribution in the brain. It was highly enriched in the basal ganglia regions (the olfactory tubercle, nucleus accumbens, caudatoputamen, globus pallidus, and substantia nigra), as we also have observed using slightly different methods (Walaas et al., 1983b, c). Nevertheless, liquid scintillation counting of gel pieces cut from the 32,000-dalton region of the gels showed ^{32}P incorporation above background in virtually all regions of the brain, including those parts where DARPP-32 could not be identified by visual inspection of the autoradiograms (Fig. 2). The extract from the anterior pituitary, a tissue which does not belong to the nervous system, did not contain any 32,000-dalton phosphoprotein. In contrast to the highly uneven distribution of DARPP-32, synapsin I was widely and relatively evenly distributed throughout the CNS. Again, the extract from the anterior pituitary did not contain measurable amounts of synapsin I, in agreement with immunocytochemical studies (De Camilli et al., 1979). Protein IIIa and protein IIIb showed distributions very similar to that of synapsin I, in agreement with earlier studies on the distribution of these proteins (Browning et al., 1982; Walaas et al., 1983a).

Figure 2 also shows the presence of an acid-soluble 62,000-dalton phosphoprotein, which had a regional distribution with certain similarities to that of DARPP-32. In particular, both proteins were highly enriched in the dopamine-innervated parts of the basal ganglia. However, the 62,000-dalton phosphoprotein was also highly concentrated in the ventral tegmental area (not shown), a region with a very low concentration of DARPP-32 (Walaas et al., 1983c). Evidence described throughout this paper indicates that the 62,000-dalton phosphoprotein quite probably represents tyrosine hydroxylase, an enzyme which is highly enriched in dopaminergic neurons (Hökfelt et al., 1976). This protein is known to be a substrate for cAMP-dependent protein kinase, the phosphorylated subunit having an apparent $M_r \sim 60{,}000$ when analyzed by SDS-PAGE (Joh et al., 1978). Therefore, we shall tentatively refer to the 62,000-dalton phosphoprotein as tyrosine hydroxylase in the remainder of this paper.

One-dimensional peptide maps after partial proteolytic digestion with *S. aureus* V8 protease were used to compare the 32,000-dalton phosphoproteins from different regions (Fig. 3). Preliminary studies showed that phosphorylated DARPP-32, whether obtained from acid extracts or from endogenously phosphorylated homogenates from rat, rabbit, or bovine caudatoputamen, always yielded the same peptide map, consisting of one major phosphopeptide fragment of 8,000 daltons and two minor fragments of 14,000 and 12,000 daltons. Similar peptide maps (Fig. 3) were obtained from all of the 32,000-dalton phosphoproteins shown in Figure 2, and liquid scintillation counting of the 8,000-dalton fragments showed ^{32}P content above background in all samples except that from the anterior pituitary (not shown). In contrast, the 62,000-dalton phosphoprotein (from the olfactory tubercle, nucleus accumbens, caudatoputamen, or substantia nigra), synapsin I, and several other phosphoproteins in the extracts gave peptide maps completely different from

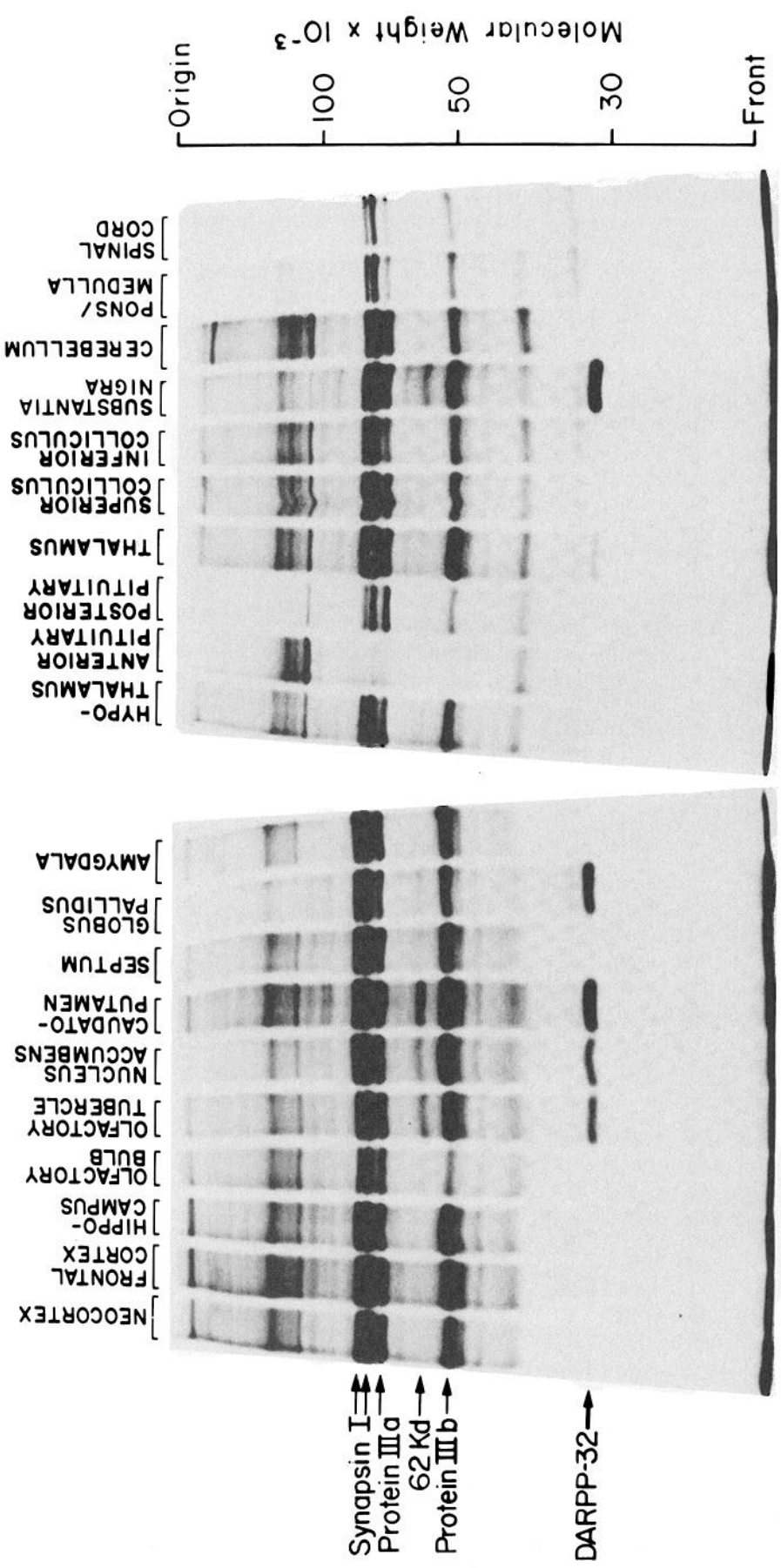

Figure 2. Autoradiograms showing the regional distribution of DARPP-32 and of synapsin I and proteins IIIa and IIIb in rat CNS. The indicated regions of rat brain, spinal cord, and pituitary gland were dissected, and acid/detergent extracts were prepared as described in the text. Aliquots containing 50 μg of protein were incubated in the presence of 10 μM [γ-^{32}P]ATP and 25 nM catalytic subunit of cAMP-dependent protein kinase for 60 min at 30°C. The phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography. Autoradiograms from a representative experiment are shown. *Arrows* indicate the localization of DARPP-32, synapsin I, protein IIIa (74,000 daltons), protein IIIb (55,000 daltons), and a 62,000-dalton (62 *Kd*) protein probably representing tyrosine hydroxylase. Gel pieces containing DARPP-32 and synapsin I were cut from the gel with the autoradiograms as guides, and ^{32}P content was measured by liquid scintillation counting. *Numbers* indicate picomoles of ^{32}P incorporated per milligram of protein in the neutralized extracts. *n.d.*, not detected (<0.05 pmol of ^{32}P/mg of protein).

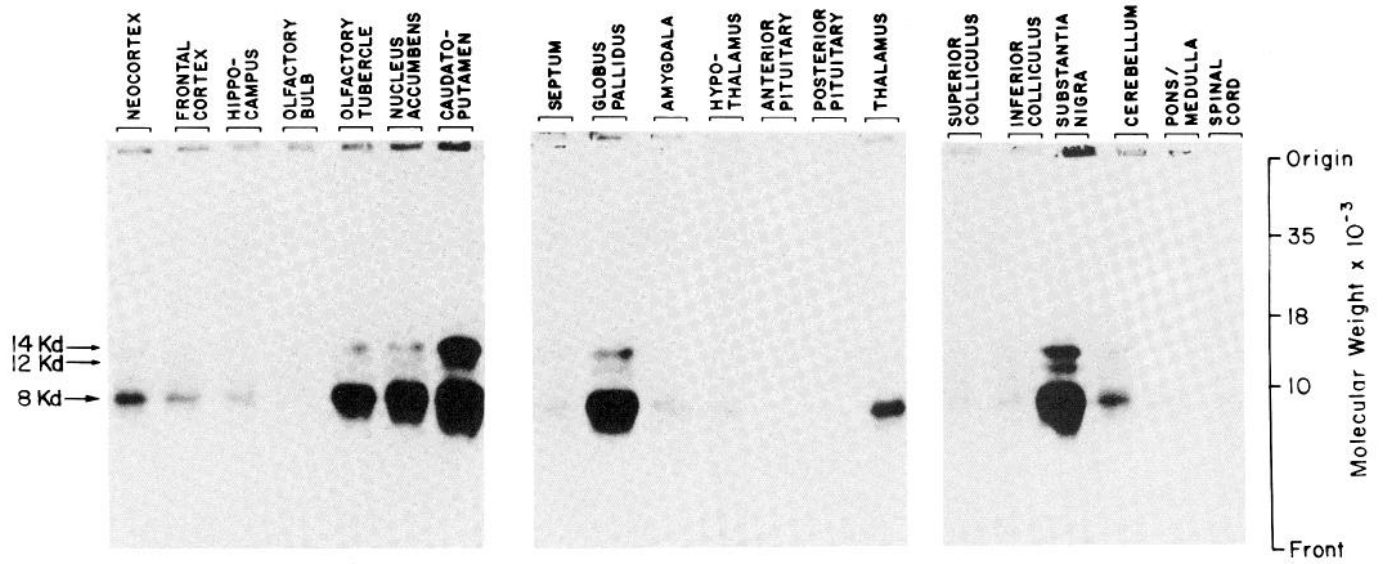

Figure 3. Autoradiograms showing one-dimensional maps of phosphopeptide fragments generated from the 32,000-dalton phosphoproteins of different regions of the rat CNS. Pieces of the gel shown in Figure 2, containing the 32,000-dalton protein, were subjected to limited proteolytic digestion with *S. aureus* V8 protease as described (Huttner and Greengard, 1979), and the phosphopeptide fragments were separated by SDS-PAGE and visualized by autoradiography.

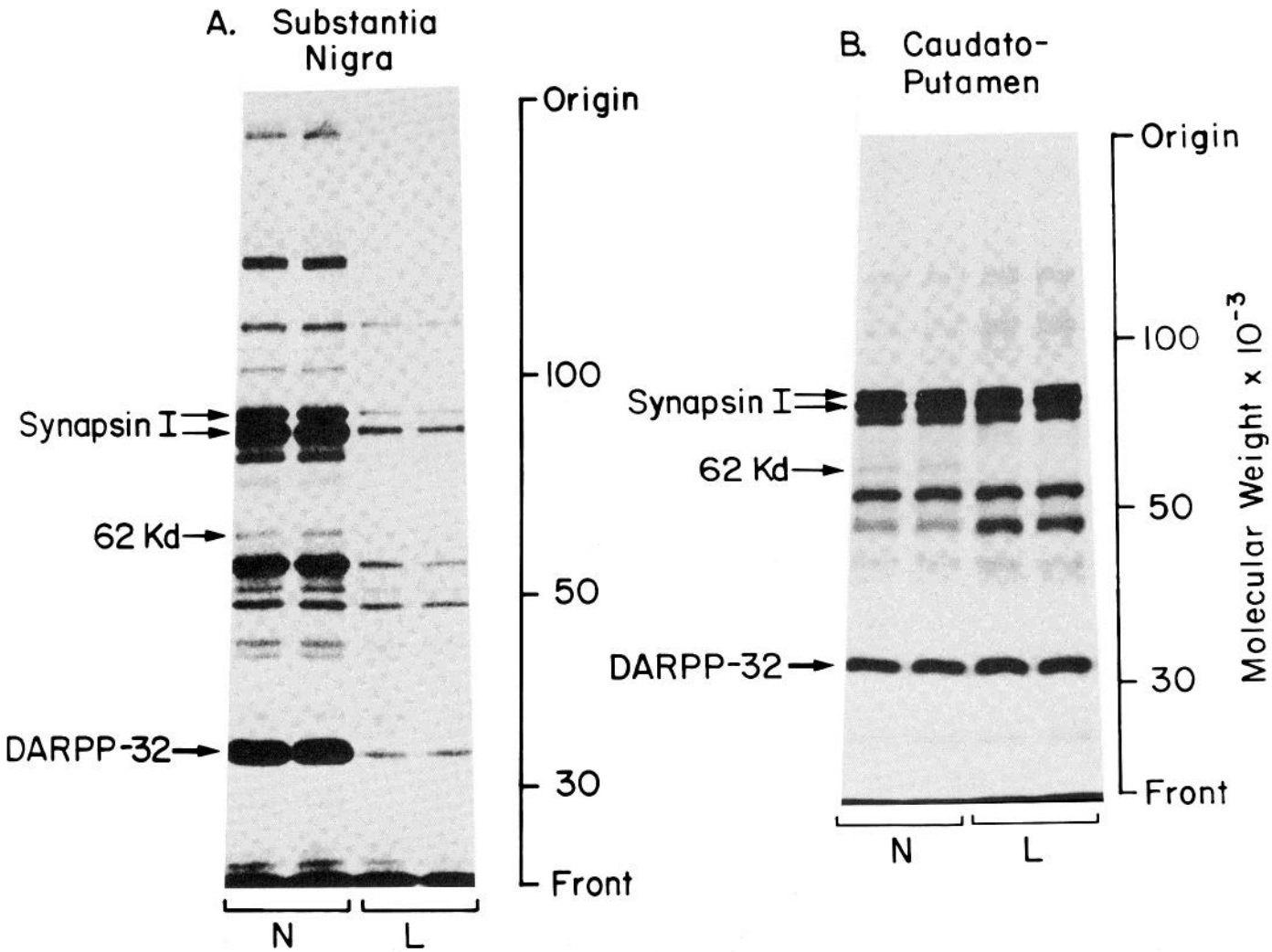

Figure 4. Autoradiograms showing the effects of brain hemitransection on the amount of acid-soluble phosphoproteins in rat substantia nigra (*A*) and caudatoputamen (*B*). Proteins present in extracts of substantia nigra and caudatoputamen from control animals (*N*) or from the lesioned side of the brains of hemisectioned animals (*L*) were phosphorylated in the presence of [γ-^{32}P] ATP and catalytic subunit of cyclic AMP-dependent protein kinase for 60 min at 30°C. Aliquots containing equal amounts of extracted proteins were subjected to SDS-PAGE, and phosphoproteins were visualized by autoradiography. Specific proteins are identified in the legend to Figure 2.

that of DARPP-32 when analyzed by the same technique (not shown).

Peripheral, non-nervous tissues were also examined for the possible presence of DARPP-32. Crude cytosol or membrane preparations from lung, heart, liver, skeletal muscle, kidney, or spleen were phosphorylated in the absence or presence of cAMP, while acid/Triton extracts prepared from the same tissues were phosphorylated in the presence of the catalytic subunit of cAMP-dependent protein kinase. No 32,000-dalton phosphoprotein was observed in any of these preparations, and peptide maps of 40,000-, 33,000-, and 30,000-dalton phosphoproteins seen in liver or kidney cytosol did not indicate any similarity between these phosphoproteins and DARPP-32 (not shown). The evidence therefore suggests that DARPP-32 may be restricted to nervous tissue.

Cellular localization of DARPP-32. The foregoing results indicated that DARPP-32 is enriched in the CNS, where the protein has a specific regional distribution and is highly concentrated in dopamine-innervated regions, particularly in the basal ganglia. The well defined neuroanatomy and neurochemistry of the basal ganglia regions (for reviews, see Nauta and Domesick, 1979; Fonnum and Walaas, 1979; Walaas, 1980) made it possible to analyze further the cellular localization of DARPP-32 in the caudatoputamen and substantia nigra, which are known to be interconnected by major nerve fiber tracts (Nauta and Domesick, 1979). Several lesion techniques were used (Fig. 1), designed to induce degeneration of either ascending nigrostriatal fibers (particularly the dopaminergic fibers (Andén et al., 1964)) or descending striatonigral fibers (which are predominantly GABAergic (for review, see Fonnum and Walaas, 1979)). Other lesions induced by either kainic acid or 6-hydroxydopamine destroyed the local neurons in the substantia nigra and caudatoputamen. The activities of neurotransmitter markers specific for dopaminergic, cholinergic, and GABAergic neurons, and the amounts of the nerve terminal marker synapsin I (De Camilli et al., 1980), were measured in some of these experiments to monitor the lesions.

In one series of experiments, we examined the effect of hemitransection of the brain, at the level of the globus pallidus, on the phosphoproteins present in the substantia nigra and caudatoputamen. This type of hemitransection destroys most of the massive projection from the striatum to the substantia nigra. The effect of brain hemitransection on phosphoproteins present in the substantia nigra is shown in Figure 4*A*: the lesion induced an almost complete disappearance of DARPP-32 and a substantial loss of synapsin I and several other phosphoproteins, including tyrosine hydroxylase. Tyrosine hydroxylase activity has in fact previously been shown to be decreased following this lesion, apparently due to retrograde degeneration (Spano et al., 1977; Quik et al., 1979). Several other phosphoproteins present in the extract were not changed by this lesion. The lesion induced a large decrease in GAD activity and no change in ChAT activity in the substantia nigra (Table I), in agreement with previous reports (Fonnum et al., 1977, 1978). In the substantia nigra, therefore, DARPP-32 appears to be highly enriched in, and may be restricted to, descending neuronal fibers. Furthermore, the considerable decrease in the level of synapsin I after this lesion indicates that the majority of nerve terminals in the substantia nigra belongs to the striatonigral fibers, supporting previous ultrastructural studies (Grofova and Rinvik, 1970).

TABLE I

Effect of brain hemitransection on phosphoproteins and neurotransmitter marker enzymes in rat substantia nigra and caudatoputamen

The amount of dephospho-DARPP-32 and dephospho-synapsin I and the activity of GAD, ChAT, and tyrosine hydroxylase in substantia nigra and caudatoputamen of the hemisectioned side of the brain were calculated as a percentage of that found in the corresponding regions of unoperated control animals. Data are expressed as mean ± SEM for six lesioned animals. Control values (mean ± SEM) in six unlesioned animals were: substantia nigra: DARPP-32, 24.7 ± 1.0 pmol/mg of protein; synapsin I, 38.8 ± 1.3 pmol/mg of protein; GAD, 480 ± 18 nmol/hr/mg of protein; ChAT, 3 ± 1 nmol/hr/mg of protein. Caudatoputamen: DARPP-32, 18.0 ± 0.4 pmol/mg of protein; synapsin I, 39.4 ± 1.2 pmol/mg of protein; GAD, 176 ± 16 nmol/hr/mg of protein; ChAT, 61 ± 7 nmol/hr/mg of protein; tyrosine hydroxylase, 1.28 ± 0.15 nmol/hr/mg of protein.

	Percentage of Unoperated Controls	
	Substantia nigra	Caudatoputamen
DARPP-32	13 ± 4[a]	90 ± 8
Synapsin I	33 ± 4[b]	74 ± 5[c]
GAD	34 ± 9[b]	95 ± 8
ChAT	112 ± 4	103 ± 4
Tyrosine hydroxylase		21 ± 2[a]

[a] $p < 0.001$ (Wilcoxon's two-sample test).
[b] $p < 0.01$ (Wilcoxon's two-sample test).
[c] $p < 0.05$ (Wilcoxon's two-sample test).

The effect of brain hemitransection on the caudatoputamen phosphoproteins is shown in Figure 4*B*. Loss of most of the nigrostriatal dopamine fibers (Andén et al., 1964) and of the thalamostriatal and amygdalostriatal fibers (Nauta and Domesick, 1979; Kelley et al., 1982) did not induce any change in the amount of DARPP-32 in the caudatoputamen. A small but reproducible decrease was seen in synapsin I (Table I). A substantial decrease in tyrosine hydroxylase occurred, as shown both by loss of the 62,000-dalton phosphoprotein (Fig. 4) and by analysis of enzyme activity (Table I), confirming that a large number of nigrostriatal fibers had degenerated. GAD and ChAT activities, present in local caudatoputamen neurons (Fonnum and Walaas, 1979), were unchanged (Table I). These results showed that removal of ascending inputs to the caudatoputamen did not affect the amount of DARPP-32 in this nucleus.

A more specific lesion of the dopaminergic nigrostriatal fibers was achieved by local infusion of 6-hydroxydopamine. This neurotoxin is known to specifically destroy catecholaminergic neurons or fibers close to the injection site (Ungerstedt, 1971; Sotelo et al., 1973). Figure 5 shows the effects of this lesion when placed in the substantia nigra. The substantia nigra itself contained virtually unchanged amounts of DARPP-32 and of synapsin I, but, as expected, showed a substantial decrease in tyrosine hydroxylase. The caudatoputamen showed an unchanged concentration of DARPP-32 (Fig. 5) but displayed a slight decrease in synapsin I and a substantial decrease in tyrosine hydroxylase. When the 6-hydroxydopamine lesion was located in the caudato-

putamen, tyrosine hydroxylase almost disappeared from the lesioned region, but no changes were observed in DARPP-32 in either the caudatoputamen or substantia nigra (not shown). The results obtained with 6-hydroxydopamine therefore indicate that DARPP-32 is located outside the dopaminergic neurons.

Finally, the local neurons in the caudatoputamen and in the substantia nigra were lesioned by local infusion of kainic acid. This neurotoxin is known to destroy dendrites, cell bodies, and axons belonging to intrinsic neurons when locally infused into brain nuclei, without lesioning glial cells, axon terminals of extrinsic neurons, or fibers passing through the lesioned regions (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976; Schwarcz and Coyle, 1977; Coyle et al., 1978). This compound, therefore, has, despite certain side effects (Nadler 1979; Zaczek et al., 1980), been used extensively to study the biochemical composition of local neurons in various parts of the brain. When kainic acid was injected into the substantia nigra (to destroy nigral neurons and efferents including the nigrostriatal fibers), no change was observed in the concentration of DARPP-32 in either the substantia nigra or the caudatoputamen (not shown). The effect of injection of kainic acid into rostral parts of the caudatoputamen (to destroy striatal neurons and striatonigral fibers (Schwarcz and Coyle, 1977; DiChiara et al., 1980)) on phosphoproteins in the substantia nigra is shown in Figure 6 and Table II: a significant decrease was seen in DARPP-32, synapsin I (Fig. 6), and GAD (Table II), but not in tyrosine hydroxylase (Fig. 6). In samples from the lesioned caudatoputamen itself, a considerable decrease in DARPP-32 was seen (Fig. 6) which was similar in magnitude to the decrease seen in the markers for local cholinergic and GABAergic neurons (Table II). The lesion also caused a significant decrease in synapsin I concentration (Table II), but no change in tyrosine hydroxylase (Fig. 6), in the caudatoputamen.

An analysis of the quantitative changes in DARPP-32, synapsin I, and the neurotransmitter marker enzymes ChAT, GAD, and tyrosine hydroxylase induced by brain hemitransection (Table I) and by kainic acid lesions of the caudatoputamen (Table II) indicates that, in the caudatoputamen, DARPP-32 is present in local neurons, while in the substantia nigra, the phosphoprotein is present in afferent fibers originating in the neostriatum. This interpretation is in agreement with results presented in the following immunocytochemical study (Ouimet et al., 1984).

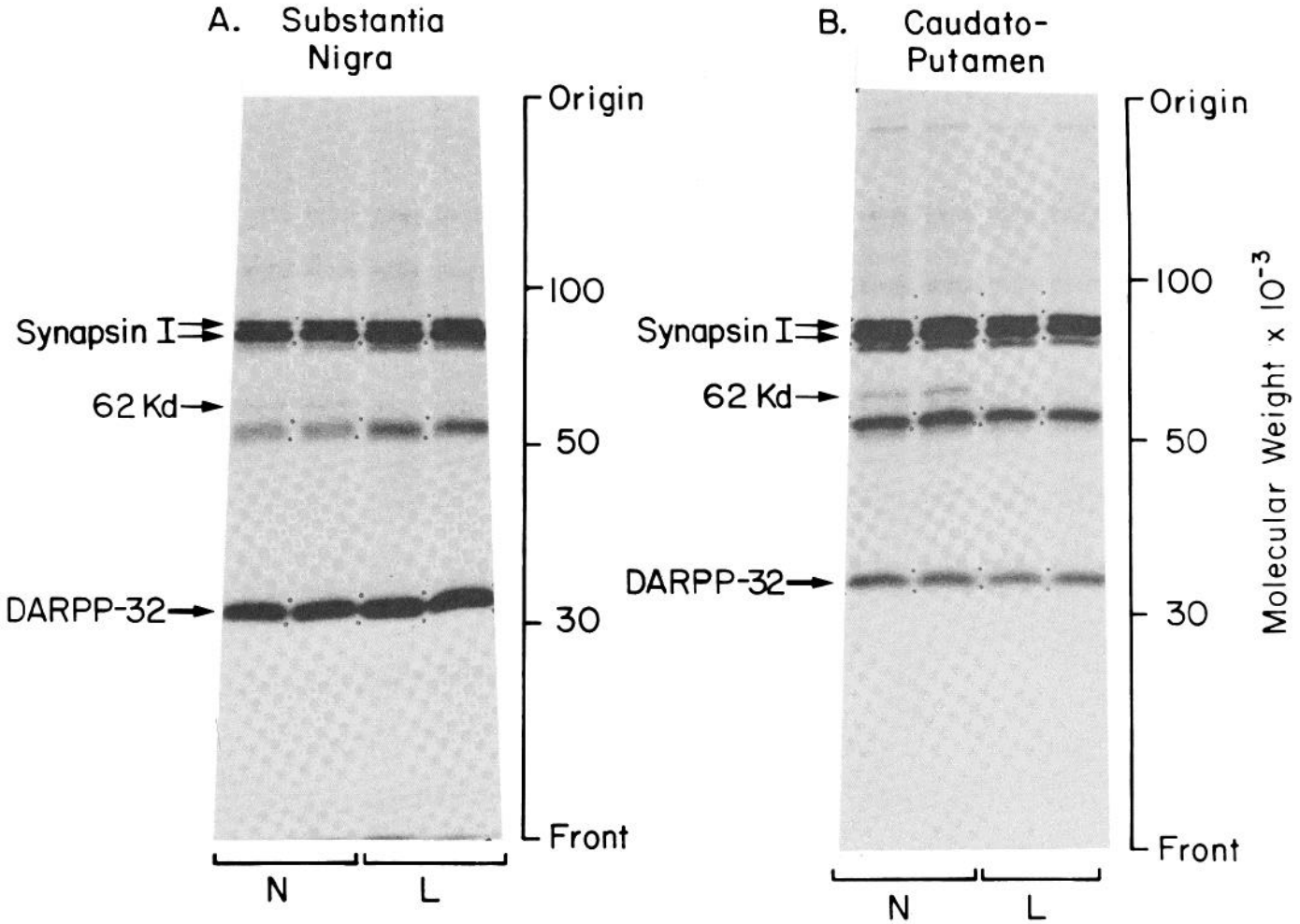

Figure 5. Autoradiograms showing the effects of local infusion of 6-hydroxydopamine (6 μg in 4 μl of KRB) into rat substantia nigra on the amount of acid-soluble phosphoproteins in substantia nigra (*A*) and caudatoputamen (*B*). Proteins present in the extracts were processed as described in the legend to Figure 4.

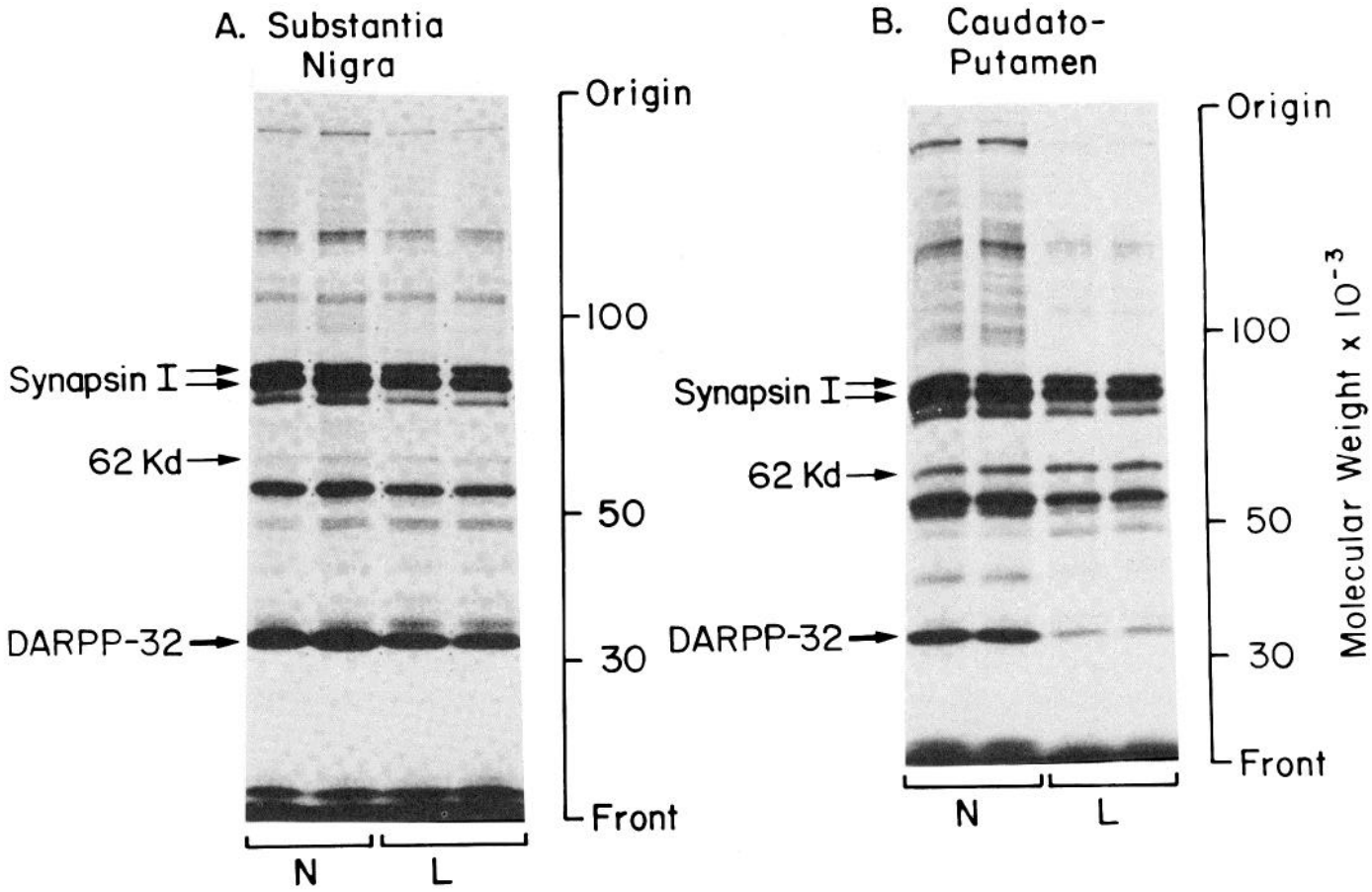

Figure 6. Autoradiograms showing the effects of local infusion of kainic acid (5 nmol) into rat caudatoputamen on the amount of acid-soluble phosphoproteins in substantia nigra (*A*) and caudatoputamen (*B*). Proteins present in the extracts were processed as described in the legend to Figure 4.

TABLE II

Effect of infusion of kainic acid into the rostral caudatoputamen on phosphoproteins and neurotransmitter marker enzymes in rat substantia nigra and caudatoputamen

Lesions were produced by infusion of 5 nmol of kainic acid into the rostral caudatoputamen rather than by hemitransection. Other details were as in the legend to Table I.

	Percentage of Unoperated Controls	
	Substantia nigra	Caudatoputamen
DARPP-32	63 ± 15[a]	30 ± 4[b]
Synapsin I	70 ± 11[a]	67 ± 4[a]
GAD	57 ± 8[b]	25 ± 7[b]
ChAT		22 ± 3[b]

[a] $p < 0.05$ (Wilcoxon's two-sample test).
[b] $p < 0.01$ (Wilcoxon's two-sample test).

Subcellular localization of DARPP-32. The foregoing results suggested that DARPP-32 is present in local neostriatal neurons and in the axons which project from the neostriatum to the substantia nigra. The subcellular distribution of the protein was therefore studied in these two brain regions. Caudatoputamen samples were separated into crude nuclear, synaptosomal, microsomal, and soluble fractions, while substantia nigra samples were separated into a particulate fraction containing nerve terminals, nuclei, and mitochondria, and a soluble fraction containing cytosol with some microsomes and small nerve terminals (Whittaker and Barker, 1972; Storm-Mathisen, 1975). Figure 7 shows the results from one such experiment. In the caudatoputamen, DARPP-32 was highly enriched in the soluble fraction (Table III). This suggests that DARPP-32 in this brain region is enriched in dendrites and cell bodies (Whittaker and Barker, 1972; Fonnum, 1972). In contrast, synapsin I was enriched in P_2 (the crude synaptosomal fraction), as were the nerve terminal markers ChAT, GAD, and tyrosine hydroxylase and the mitochondrial marker fumarase (Table III). A small amount of DARPP-32 was also observed in the crude synaptosomal fraction (Fig. 7). After hypotonic lysis of this fraction and centrifugation, both DARPP-32 and tyrosine hydroxylase were found in the synaptosomal cytosol, whereas synapsin I sedimented with the synaptosomal pellet (not shown).

In the substantia nigra, DARPP-32 was enriched in the particulate fraction (Fig. 7) which contained most of the nerve terminals, as indicated by synapsin I and by GAD activity (Table IV). Tyrosine hydroxylase was found in the soluble fraction, in agreement with its known localization in dopaminergic cell bodies and dendrites in this region (Hökfelt et al., 1976). Hypotonic lysis of the substantia nigra nerve terminals and centrifugation again indicated that DARPP-32 in the nerve terminals was present in the cytosol (not shown).

Regulation of DARPP-32 phosphorylation in cell-free

preparations. We have previously shown that incubation of cytosol from caudatoputamen with [γ-^{32}P]ATP plus Mg^{2+} in the presence of cAMP plus IBMX stimulated endogenous phosphorylation of DARPP-32 and of a number of other proteins, some of which have a restricted regional distribution (Walaas et al., 1983b). The phosphorylation of DARPP-32 in this preparation was rapid: the ^{32}P content of the protein was half-maximal after 5 to 10 sec of incubation, reached maximal levels after 60 to 120 sec, and declined thereafter (not shown). In contrast, other cytosolic phosphoproteins exhibited a distinctly slower rate of ^{32}P incorporation under these experimental conditions (not shown).

Incubation of cytosol preparations from the caudato-

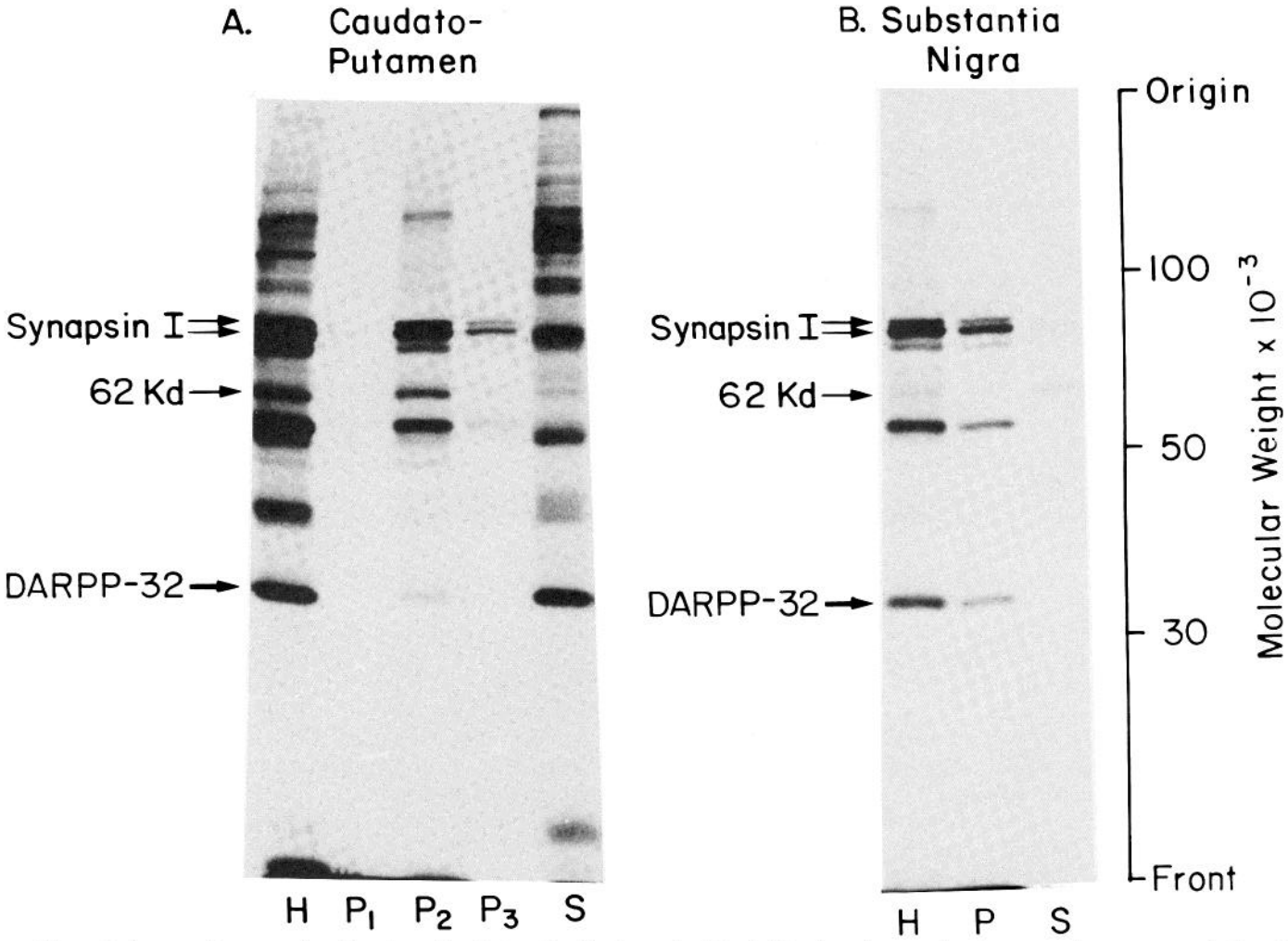

Figure 7. Autoradiograms showing the subcellular distribution of acid-soluble phosphoproteins in rat caudatoputamen (*A*) and substantia nigra (*B*). Caudatoputamen homogenates (*H*) were separated into crude nuclear (P_1), synaptosomal (P_2), microsomal (P_3), and cytosolic (*S*) fractions; substantia nigra homogenates (*H*) were separated into particulate (*P*) and soluble (*S*) fractions. All fractions were subjected to the standard extraction procedure. Proteins present in the extracts were processed as described in the legend to Figure 2. Aliquots representing equal amounts of original tissue wet weight were separated by SDS-PAGE and visualized by autoradiography. Results from a representative experiment are shown.

TABLE III

Subcellular distribution of phosphoproteins and neurotransmitter marker enzymes in rat caudatoputamen

Results from a typical experiment are shown. Activities represent the mean of duplicate determinations and are expressed as a percentage of the corresponding activity present in the homogenate. Values in homogenate preparations are: fumarase, 93 units/mg of protein; lactate dehydrogenase (LDH), 834 nmol/min/mg of protein; others are given in the legend to Table I.

Fraction	DARPP-32	Synapsin I	ChAT	GAD	Tyrosine Hydroxylase	Fumarase	LDH	Protein
Crude nuclear (P_1)	5	6	6	12	2	11	3	9
Synaptosomal (P_2)	16	64	66	46	52	67	42	46
Microsomal (P_3)	5	18	15	15	6	10	12	15
Soluble (S)	76	ND[a]	11	46	26	10	44	27
Recovery	102	88	98	119	86	98	101	97

[a] ND, not detected.

putamen or from other DARPP-32-enriched regions in the presence of cGMP plus IBMX (not shown), or of calcium in the absence or presence of calmodulin or phosphatidylserine (not shown), did not stimulate the phosphorylation of DARPP-32. This lack of effect of calcium was not due to inactive calcium-dependent protein phosphorylation systems, since a great number of other proteins were phosphorylated by endogenous calcium-dependent protein kinases (Walaas et al., 1983b). Thus, DARPP-32 in cell-free preparations is a good substrate for endogenous cAMP-dependent protein kinase, but not for calcium/calmodulin-dependent or calcium/phospholipid-dependent protein kinases.

Regulation of DARPP-32 phosphorylation in intact cells. To study the state of phosphorylation of DARPP-32 in intact cells, slices of the caudatoputamen were incubated under conditions which have been found to selectively stimulate either cAMP-dependent or calcium-dependent protein kinase activity in brain slices (Forn and Greengard, 1978). Incubation of these slice preparations with 8-bromo-cAMP (4 mM for 10 min) induced a significant decrease in the amount of dephospho-DARPP-32 (Table V), which ranged in various experiments from 25% to 50%. Since the total amount of DARPP-32 remained constant during the incubation of the tissue, the results demonstrate that 8-bromo-cAMP increased the state of phosphorylation of DARPP-32 in these slices. A substantial decrease in the amount of dephospho-synapsin I was also observed in these experiments (Table V), and qualitatively similar results were observed with proteins IIIa and IIIb (not shown).

In other experiments, slices were incubated with IBMX (1 mM for 10 min). This potent inhibitor of cyclic nucleotide phosphodiesterase has been found to induce a maximal phosphorylation of synapsin I in brain slices, probably acting through an increase in cAMP levels, and thereby activating cAMP-dependent protein kinase (Forn and Greengard, 1978). This treatment induced a small, but significant decrease in the amount of dephospho-DARPP-32 (Table V), which ranged in magnitude between 10% and 20%. In contrast, about 50% of synapsin I in the caudatoputamen slices was consistently phosphorylated in response to this treatment.

Incubation of the caudatoputamen slices with depolarizing concentrations of KCl (60 mM for 30 sec) did not change the state of phosphorylation of DARPP-32 (Table V). In contrast, there was a marked decrease in the amount of dephospho-synapsin I and dephospho-proteins IIIa and IIIb under the same conditions, in agreement with earlier studies using brain slices (Forn and Greengard, 1978). KCl-induced depolarization, which induces calcium influx into neurons through voltage-sensitive calcium channels (Blaustein, 1975; Krueger et al., 1977), has been shown to activate both calcium/calmodulin-dependent and calcium/phospholipid-dependent protein kinases in intact brain cells and nerve terminals (Krueger et al., 1977; Forn and Greengard, 1978; Wu et al., 1982).

TABLE IV

Subcellular distribution of phosphoproteins and neurotransmitter marker enzymes in rat substantia nigra

Results from a typical experiment are shown. Activities represent the mean of duplicate determinations and are expressed as percentage of the corresponding activity present in the homogenate. Values in homogenate preparations are: tyrosine hydroxylase, 0.36 nmol/hr/mg of protein; others are given in the legend to Table I.

	DARPP-32	Synapsin I	GAD	Tyrosine Hydroxylase	Protein
P	63	63	62	8	77
S	26	15	39	97	35
Recovery	89	78	101	105	112

TABLE V

The effect of 8-bromo-cAMP, IBMX, and KCl on phosphorylation of DARPP-32 and synapsin I in rat caudatoputamen slices

Caudatoputamen slices were incubated in the presence of 4 mM 8-bromo-cAMP or 1 mM IBMX for 10 min, or 60 mM KCl for 30 sec. The amount of dephospho-DARPP-32 and of dephospho-synapsin I in experimental slices were calculated as a percentage of that in contralateral control slices (slices incubated in the absence of test substances). Data are expressed as mean ± SEM for the number of experiments shown in parentheses.

Test Substance	Percentage of Contralateral Control	
	Dephospho-DARPP-32	Dephospho-Synapsin I
4 mM 8-Bromo-cAMP	49 ± 2 (4)[a]	33 ± 2 (4)[a]
1 mM IBMX	86 ± 4 (4)[b]	51 ± 1 (4)[a]
60 mM KCl	98 ± 4 (14)	44 ± 3 (14)[a]

[a] $p < 0.01$ compared to control samples (Wilcoxon's paired comparison test).

[b] $p < 0.05$ compared to control samples (Wilcoxon's paired comparison test).

The studies on the regional distribution of DARPP-32 described above indicated that this phosphoprotein is highly enriched in those regions of the brain containing dopaminergic nerve terminals. The lesion studies carried out to determine the cellular localization of DARPP-32 indicated that this protein was present in dopaminoceptive but not in dopaminergic neurons. It was, therefore, of interest to examine the possible regulation by dopamine of DARPP-32 phosphorylation in intact cells of the caudatoputamen. In initial studies (Walaas et al., 1983c) it was found that incubation of slices with dopamine (100 μM for 10 min), in the presence of a low concentration of IBMX (20 μM), induced a significant decrease in the amount of dephospho-DARPP-32. In the present studies we have examined the effect of varying the concentration of dopamine (Table VI) and the incubation time (Table VII) on the amount of dephospho-DARPP-32 present in rat caudatoputamen slices. Maximal effects were observed at 100 μM dopamine (Table VI) and at 5 min incubation time (Table VII). Since the total amount of DARPP-32 remained constant during the incubation of the tissue, the results demonstrate that dopamine increased the state of phosphorylation of DARPP-32 in these slices.

In one series of experiments, we examined the effect of fluphenazine, a potent antipsychotic dopamine receptor blocker, on the decrease in the amount of dephospho-DARPP-32 induced by incubation of slices with 100 μM dopamine for 10 min. In these experiments, fluphenazine was added to the medium 5 min before the addition of dopamine. A half-maximal inhibition of the dopamine

TABLE VI

The effect of various concentrations of dopamine on DARPP-32 phosphorylation in rat caudatoputamen slices

Caudatoputamen slices were incubated for 10 min with the indicated concentrations of dopamine. The amount of dephospho-DARPP-32 in experimental slices was calculated as a percentage of that in contralateral control slices (slices incubated in the absence of dopamine). Data are expressed as mean ± SEM for the number of experiments shown in parentheses.

Dopamine Concentration ($M \times 10^{-6}$)	Dephospho-DARPP-32 (% contralateral control)
1	98 ± 7 (3)
20	77 ± 9 (4)[a]
100	66 ± 4 (6)[b]
200	64 ± 4 (4)[b]
1000	75 ± 3 (4)[a]

[a] $p < 0.05$ (Wilcoxon's paired comparison test).
[b] $p < 0.01$ (Wilcoxon's paired comparison test).

TABLE VII

The effect of dopamine on DARPP-32 phosphorylation in rat caudatoputamen slices as a function of incubation time

Caudatoputamen slices were incubated in the presence of 100 μM dopamine for the indicated periods of time. The amount of dephospho-DARPP-32 was calculated as a percentage of that in contralateral control slices (slices incubated in the absence of dopamine). Data are expressed as mean ± SEM for the number of experiments shown in parentheses.

Incubation Time (min)	Dephospho-DARPP-32 (% contralateral control)
0.5	95 ± 6 (4)
1	81 ± 9 (6)[a]
2	81 ± 6 (6)[a]
5	57 ± 3 (6)[b]
10	66 ± 4 (6)[b]

[a] $p < 0.05$ (Wilcoxon's paired comparison test).
[b] $p < 0.01$ (Wilcoxon's paired comparison test).

effect was observed at 5×10^{-7} M fluphenazine (data not shown).

Incubation of caudatoputamen slices with 100 μM norepinephrine or 100 μM serotonin for 10 min did not affect the state of phosphorylation of DARPP-32. Although norepinephrine is highly effective in inducing cAMP formation in caudatoputamen slices through activation of β-adrenergic receptors (Palmer et al., 1973; Forn et al., 1974), this effect appears to take place in glial cells (Minneman et al., 1978).

Discussion

The present study has described DARPP-32, a phosphoprotein in mammalian brain which may play a biological role in dopaminoceptive neurons. DARPP-32 has a number of properties which suggest such a role. The protein can be phosphorylated in slice preparations containing intact cells by the application of dopamine in concentrations which activate specific dopamine receptors linked to adenylate cyclase (Forn et al., 1974), and a similar phosphorylation can be induced by the extracellular application of 8-bromo-cAMP. In cell-free preparations, DARPP-32 is a substrate for endogenous cAMP-dependent protein kinase and protein phosphatase. The regional distribution of the protein in the brain follows the general pattern of dopaminergic innervation. Lastly, lesion studies in two of these dopamine-innervated brain regions demonstrate that the protein is not present in the dopamine-containing neurons themselves, but rather in neurons which have been reported to possess dopamine receptors linked to adenylate cyclase activation, and which appear to be dopaminoceptive (Kebabian and Calne, 1979; Fonnum and Walaas, 1979). Therefore, the phosphorylation of DARPP-32 may be regulated by synaptically released dopamine, and the phosphoprotein may be involved in mediating or modulating dopamine-induced trans-synaptic effects achieved through the dopamine receptor which is linked to adenylate cyclase activation (the D-1 receptor).

The specific regional distribution of DARPP-32 found in the present study suggests a link to the dopaminergic neurotransmitter system. The considerable enrichment of the protein in the substantia nigra, where the most important dopaminergic fiber system in the mammalian brain originates (Lindvall and Björklund, 1978), and in the neostriatum, where most of this dopaminergic system terminates, contrasts strongly with the low concentration of DARPP-32 in regions which have a low density of dopamine fibers, but are innervated by other neurotransmitters known to regulate cAMP synthesis, such as norepinephrine or serotonin. For example, the lower brainstem, where both serotonergic and noradrenergic cell groups are located (Dahlström and Fuxe, 1964), and the hypothalamus and hippocampus, where serotonin and norepinephrine terminals are concentrated (Versteeg et al., 1976; Azmitia and Segal, 1978), contain little DARPP-32.

In certain instances, the regional distribution of DARPP-32 determined by microdissection and that of dopaminergic innervation did not correlate well. For example, the concentration of DARPP-32 is much lower in the amygdala than in the basal ganglia, even though the amygdala has a distinct dopaminergic input (Lindvall and Björklund, 1978). However, the immunocytochemical studies on DARPP-32 presented in an accompanying paper (Ouimet et al., 1984) demonstrate the presence of small populations of DARPP-32-immunoreactive cells in precisely those parts of the amygdala where a dopaminergic input has been described (Fallon et al., 1978; Lindvall and Björklund, 1978). A similar specific regional correlation appears to exist in the hypothalamus as well (Ouimet et al., 1984).

Some characteristics of the regional distribution of DARPP-32 indicate that the protein is concentrated in a subpopulation of dopaminoceptive cells, namely, those containing D-1 receptors. In contrast, no evidence was obtained for the presence of DARPP-32 in the subpopulation of dopaminoceptive neurons containing D-2 receptors (dopamine receptors not linked to adenylate cyclase activation (Kebabian and Calne, 1979)). For example, both DARPP-32 and D-1 receptors may be present in the same population of local cells in the caudatoputamen. Thus, we observed a large decrease in DARPP-32 concentration in this nucleus after kainic acid infusion, a decrease which was similar in magnitude to the decrease of D-1 receptors which followed this lesion (McGeer et al., 1976; Schwarcz and Coyle, 1977; Cross

and Waddington, 1981). In contrast, the D-2 receptors in the caudatoputamen are at least partly located on both corticostriatal and nigrostriatal afferent fibers (Schwarcz et al., 1978; Murrin et al., 1979), which are not enriched in DARPP-32 (this paper, and unpublished observations). In the substantia nigra, DARPP-32 is confined to the striatonigral fibers and terminals, and is not found in local cells. This localization is again similar to that of the D-1 receptors (Premont et al., 1976; Phillipson et al., 1977; Spano et al., 1977; Quik et al., 1979). D-2 receptors are absent from the striatonigral fibers but are present on local neurons in the substantia nigra (Murrin et al., 1979; Quik et al., 1979). In addition, DARPP-32 is present only in trace amounts in the ventral tegmental area (Walaas et al., 1983c) and is virtually absent from the anterior pituitary, two regions containing D-2 but not D-1 receptors (Premont et al., 1976; Kebabian and Calne, 1979; Gundlach et al., 1982). Finally, there is a good correlation between the distribution of DARPP-32 and D-1 receptors in other regions of the brain, as discussed in the accompanying immunocytochemical paper (Ouimet et al., 1984). The regional and cellular localization of DARPP-32 thus shows that this phosphoprotein has a highly specific brain localization, which in most regions seems to be related to the presence of D-1 receptors.

cAMP appears to be the intracellular messenger which regulates the phosphorylation of DARPP-32. cAMP has previously been found to mimic certain electrophysiological actions of dopamine on caudatoputamen neurons (Siggins et al., 1974). Further studies will be required to determine whether there is a relationship between these biochemical and electrophysiological responses to dopamine and cAMP. Any model of a role for DARPP-32 should take into account its cytosolic localization and its distribution not only in striatal cell bodies and dendrites but also in the descending striatonigral fibers and nerve terminals.

In conclusion, the present study documents the existence of a brain phosphoprotein associated with a subpopulation of dopaminoceptive neurons containing D-1 receptors. The precise functional role of this type of dopamine receptor has remained difficult to define in the brain (see, e.g., Euvrard et al., 1979; Bockaert, 1981). Hopefully, an increased understanding of the biological role of DARPP-32 may also lead to a greater understanding of those functions of dopamine mediated by the D-1 receptor.

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