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Modulation of brain catecholamine absorbing proteins by dopaminergic agents

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Abstract

Catecholamine absorbing proteins (CATNAPs) are localized in the brain and thus far have no known biochemical and pharmacological characteristics consistent with other receptor proteins or metabolic enzymes in the central nervous system. The oxidative metabolism of catecholamines in the brain, especially the catabolism of dopamine and its conjugation with metabolic brain proteins, results in the production of highly toxic free radicals. Since such processes are implicated in the pathophysiology of various neurodegenerative diseases, including parkinsonism, and since CATNAPs bind catecholamines with high affinity, there is a need to further investigate if these novel proteins could play a protective role against these harmful catecholamine metabolites. In this study, we demonstrate the purification, pharmacological characterization and modulation of CATNAPs, as the first steps necessary to elucidate the function of these proteins in the brain. First, CATNAPs were identified from tissues using [^{3}H]*N*-*n*-propylnorapomorphine (a specific dopamine receptor agonist) and [^{125}I]6-hydroxy-5-iodo[*N*(*N*-2,4-dinitro-phenyl)-aminopropyl]1,2,3,4-tetrahydronaphthalene ([^{125}I]DATN; a highly specific ligand synthesized in our laboratory). Three proteins, with molecular masses of 47, 40 and 26 kDa, were identified and purified, which allowed for the subsequent production of antibodies against each of these CATNAPs. The effects of in vivo chronic administration of several dopaminergic agents on CATNAPs were also examined by Western immunoblotting. L-3,4-Dihydroxyphenylalanine (L-DOPA) treatment in rats resulted in the increase of all of the three proteins, as compared to controls. Treatment in rats with the dopamine depleting agent, reserpine, produced a significant decrease in all of the three CATNAPs. In addition, the effects of direct administration of apomorphine, dopamine, epinephrine, isopropylnorepinephrine, norepinephrine, *N*-*n*-propylnorapomorphine and 6-hydroxydopamine on CATNAP levels in rats were examined. Interestingly, we observed an increase (as compared to control) of the 47, 40 and 26 kDa proteins in animals treated with dopamine, norepinephrine, *N*-*n*-propylnorapomorphine and apomorphine. In contrast, animals treated with 6-hydroxydopamine showed significant decreases in the levels of all three proteins. It is evident that as the concentration of catecholamines increases, there is a corresponding increase in the levels of CATNAPs in the brain. These results clearly demonstrate the pharmacological modulation of CATNAPs by dopaminergic agents and suggest their possible role in the cytoprotection against damage caused by free radicals generated by oxidative stress.

Keywords: Catecholamine absorbing protein; Central nervous system; Free radical; Oxidation; Dopamine; Neurodegeneration; Parkinson's disease

1. Introduction

Catecholamines act on their target cells by binding rapidly and reversibly to specific cell surface receptor proteins (Seeman, 1980, 1994; Dohlman et al., 1991). In addition to their membrane bound receptors, dopaminergic catecholamines such as dopamine, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN), *N*-*n*-propylnorapomorphine and apomorphine are known to interact with several proteins that are found in the central nervous system. There is currently a great deal of information on the biochemistry, pharmacology and distribution of these central nervous system proteins (Niznik and Van Tol, 1992), which are involved in the metabolism of dopamine and include a presynaptic autoreceptor, dopamine transporter, and dopamine uptake sites.

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In addition, dopamine and related catecholamines interact with several metabolic enzymes, including catechol-*O*-methyltransferase, monoamine oxidases and tyrosine hydroxylase. Catechol-*O*-methyltransferase, which is widely distributed in high concentrations in the brain, as well as other tissues (Axelrod, 1957), is involved in the catabolism of dopamine and other catecholamines, such as apomorphine (Sourkes and Lal, 1975; Gao et al., 1990). Monoamine oxidases, which also oxidize dopamine, exist as two distinct subtypes: monoamine oxidase-A and monoamine oxidase-B (Miramiura and Yasunobu, 1978). The ability of tyrosine hydroxylase to convert L-tyrosine into L-3,4-dihydroxyphenylalanine (L-DOPA) is also regulated by the concentration of dopamine and other catecholamines (Hoeldtke and Kaufman, 1977).

Furthermore, catecholamines in the central nervous system can also covalently bind to the products of dopamine metabolism (Saner and Thoenen, 1971; Maguire et al., 1974; Scheulen et al., 1975; Rotman et al., 1976; Kato et al., 1986). The biochemical processes of either enzymatic or non-enzymatic monoamine catabolism can lead to the production of free radical species, which are highly toxic to cells (Cohen, 1983; Cadet, 1993; Coyle and Puttfarcken, 1993; Olanow, 1993). Dopamine, in particular, has been shown to undergo oxidative reactions which can lead to the formation of cysteinyl-dopamine and cysteinyl-3,4-dihydroxyphenylacetic acid (catechol-protein conjugates) after incubation of striatal slices with [^{3}H]dopamine (Hastings and Zigmond, 1994). These investigators have also shown that in vivo intrastriatal injections of dopamine (1.0 μmol in 2 μl) lead to the formation of cysteinyl-dopamine conjugates, which they suggested might play a role in several pathophysiological conditions. Beaudet and Descarries (1984) reported concentrations of norepinephrine in synaptic vesicles to be 10 mM, while dopamine concentrations were as high as 0.1–1 mM (Lichtensteiger, 1970; Jonsson, 1971). Michel and Kefti (1990) showed that such high concentrations may result in toxicity unless regulated by protective mechanisms and findings from several studies now indicate that free radicals generated during catecholamine metabolism play an important role in the pathogenesis of neurodegenerative disorders (Olanow, 1992; Cadet, 1993; Coyle and Puttfarcken, 1993; Olanow, 1993; Olanow and Perl, 1993; Youdim et al., 1993; Dexter et al., 1994; Jenner, 1994).

Recently, we have described a new class of central nervous system proteins called catecholamine absorbing proteins (CATNAPs), which have molecular masses of 47, 40 and 26 kDa, and are capable of covalently coupling to the catecholamines, [^{3}H]dopamine, [^{3}H]ADTN and [^{3}H]*N*-*n*-propylnorapomorphine, when incubated in vitro with synaptosomal/mitochondrial membrane preparations (Ross et al., 1993, 1995). These proteins do not exhibit biochemical, pharmacological or distribution characteristics consistent with known dopamine receptor proteins or metabolic enzymes in the central nervous system. Furthermore, they do not exhibit the pharmacological characteristics consistent with other receptor systems that interact with apomorphine, including adrenergic (Cools, 1980), serotonergic (Lee and Geyer, 1984) and opiate (Szechtman, 1986) receptors.

The ability of CATNAPs to covalently bind catecholamines (Ross et al., 1993, 1995) has provided a novel mechanism, whereby reactive compounds (e.g. quinones and semiquinone radicals) arising from oxidative events could be scavenged by specific central nervous system proteins. In the present study we describe the pharmacological effects of L-DOPA, catecholamines, and catecholamine depleting agents on CATNAPs in the striatum. The results, obtained by using highly specific antibodies, demonstrate the modulation of CATNAPs by pharmacological treatment with dopaminergic agents and suggest their role in cytoprotective processes against the neuronal injury caused by free radicals.

2. Materials and methods

Dopamine, *N*-*n*-propylnorapomorphine, apomorphine, epinephrine, norepinephrine, isopropylnorepinephrine, L-DOPA, 6-hydroxydopamine and reserpine were all purchased form Research Biochemicals International (Natick, MA, USA). [^{125}I]Na (2 mCi) was purchased from the Department of Radiopharmaceuticals, McMaster University (Hamilton, Ontario, Canada). Compounds used for displacement studies were purchased from Research Biochemicals International (Natick, MA, USA) or Aldrich Chemical Co. (Milwaukee, WI, USA). Electrophoresis reagents were obtained from Bio-Rad Laboratories (Mississauga, Ontario, Canada). Other biochemical reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Male Sprague-Dawley rats (300–350 g) and New Zealand white rabbits (2–3 kg) were obtained from Charles River Breeding Laboratories (Canada). Bovine brains were obtained from a local abattoir and other brain tissues from Pel-Freeze (Rogers, AR, USA). All tissues were stored at −70°C until required, at which time they were thawed on ice.

2.1. Synthesis of compound-A: (6-hydroxy-5-iodo[N(N-2,4-dinitrophenyl)aminopropyl] 1,2,3,4-tetrahydronaphthalene ([^{125}I]DATN)

Synthesis of [^{125}I]DATN is described in detail by Mishra et al. (1992) and Ross et al. (1995).

2.2. Labelling of membrane preparation with [^{125}I]DATN, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The crude synaptosomal/mitochondrial membranes were prepared by homogenizing the tissue in a Polytron

homogenizer (setting 6, for 20 s), in 20 volumes of buffer (50 mM Tris-HCl, 5 mM ethylene-diaminetetra-acetic acid disodium salt (EDTA), pH 7.4). The membrane suspension was subsequently centrifuged for 10 min at 50 000 × *g*, the pellet resuspended in the same buffer and centrifuged again. The pellet was then resuspended in buffer (50 mM Tris-HCl, pH 7.4), to give a final protein concentration of approximately 1 mg/ml. Protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. For labelling with [^{125}I]DATN, each assay tube contained 200 μg protein, in 50 mM Tris-HCl buffer, pH 7.4 and 1 nM [^{125}I]DATN (2200 Ci/mM specific activity) in a total assay volume of 1 ml. Samples were incubated in the dark, at 25°C for 30 min, centrifuged for 10 min at 15 000 × *g* and the supernatant discarded. The pellet was then processed for SDS-PAGE (Laemmli, 1970) using 12% slab gels under reducing conditions. Following SDS-PAGE, gels were stained with either Coomassie blue or with silver staining, processed for fluorography (Bonner, 1984), and exposed to film (Kodak X-OMAT-XAR 5) at −70°C. Under these conditions, exposure times of 7–10 days were required, after which the film was processed manually.

2.3. Purification of 47, 40 and 26 kDa proteins

The electroelution of protein was carried out using a Bio-Rad Model 422 Electroelution Apparatus (Mississauga, Ontario, Canada). After separation of proteins by SDS-PAGE, the gels were stained with Coomasie blue or 10% potassium hydroxide and the corresponding 47, 40 and 26 kDa protein bands visible on the gel were then cut and loaded into the electroelution apparatus. The top and bottom chambers of the electroelution units were filled with elution buffer containing 0.1% sodium dodecyl sulfate in 50 mM ammonium bicarbonate. Electroelution was carried out at 12–15 mA for 3–5 h, with vigorous stirring throughout. At the end of the run, the concentrated protein that had collected in the membrane caps was removed and stored in glass vials at 0°C to precipitate out salts. These protein solutions were filtered through 0.45 μm filters and finally stored at −20°C.

2.4. Characterization of 47, 40 and 26 kDa proteins using high performance liquid chromatography (HPLC)

The concentrated protein samples collected after electroelution were characterized using a Biosil-125 (0.5 μm, 0.5 × 26 cm) gel filtration column (Bio-Rad, Mississauga, Ontario, Canada). The HPLC column was pre-calibrated using Bio-Rad gel filtration standards, having molecular weights of 286 000, 156 000, 45 000, 17 000 and 1200 kDa. Approximately 20 μl of concentrated sample was injected into the HPLC and the column was eluted (flow rate of 1.0 ml/min) using a phosphate buffer mobile phase (0.05 M sodium dihydrogen orthophosphate, 0.05 M di-sodium hydrogen orthophosphate, 0.01 M sodium azide, pH 7.4). The fractions corresponding to molecular weights of 47, 40 and 26 kDa were collected and concentrated using a Minicon-B15 protein concentrator (Amicon, Beverly, MA, USA). The purified protein fractions were analyzed by SDS-PAGE and the single bands obtained at the position corresponding to the 47, 40 and 26 kDa positions were indications of purity.

2.5. Antibody production

300 μg of the purified 47, 40 and 26 kDa protein preparations were used for immunization of New Zealand white rabbits (2–3 kg). Each protein sample was mixed with 3 ml of Freund's complete adjuvant (Gibco BRL, Burlington, Ontario, Canada) and injected subcutaneously at multiple sites. Two booster injections (21 days apart) were given, with the same amount of antigen suspended in Freund's incomplete adjuvant (Gibco BRL, Burlington, Ontario, Canada). Seven days following the last injection blood samples were collected from the ear vein. The titres of anti-47, -40 and -26 kDa antibodies were determined by enzyme-linked immunosorbent assay (ELISA), and the specificity of each antibody was established by Western blotting.

2.6. Purification of immunoglobulin G (IgG) molecules

Purification of IgG by affinity chromatography was carried out according to the method of Kasper and Hartman (1987). Blood was allowed to clot overnight at 4°C and the serum was collected after centrifugation at 3000 × *g* and then stored at −20°C. For purification of the IgG fraction, a 3 ml aliquot of serum was centrifuged for 20 min at 10 000 × *g* and the supernatant collected. To this supernatant, 1 ml of 0.05 M phosphate buffered saline (PBS) was added and the mixture was passed through a Protein A-Sepharose column (8 × 1.25 cm) equilibrated with 0.1 M PBS, pH 8.2 and washed thoroughly with the same buffer. The absorbance profile was followed using an ultraviolet monitor (Pharmacia, Upsala, Sweden). After elution of unwanted serum proteins, IgG was eluted with an acidic solution (2.5 ml glacial acetic acid, 4.5 g sodium chloride, 0.2 g sodium azide in 500 ml distilled water, pH 4.0). 1 ml fractions were collected in tubes containing 0.2 ml of 0.5 M PBS, pH 8.2. Fractions were collected until the absorbance values returned to baseline. Desired fractions were pooled, the pH was adjusted to 7.0 with alkaline PBS and stored at −20°C.

2.7. Immunoblotting

Upon completion of SDS-PAGE, proteins were transferred electrophoretically to a nitrocellulose membrane using a Transblot cell (Millipore, Mississauga, Ontario, Canada) as described by Towbin et al. (1979). After

electrophoretic transfer, the nitrocellulose membranes were blocked with 5% skim milk powder in buffer consisting of 0.2% Tween-20 in Tris-buffered saline (pH 7.4) for 2 h. Transblots were then labelled with a primary anti-47, -40 and -26 kDa antibody (1 : 1000 dilution) for 2 h at room temperature, and subsequently washed 3 times with the above buffer. The membrane was then incubated with HRP-conjugated second antibody (donkey anti-rabbit, 1 : 4000 dilution) for 2 h, and washed 3 times with the same buffer. Desired protein bands were detected using either enhanced chemiluminescence (ECL) (Boehringer Mannheim, Laval, Quebec, Canada) with Kodak X-OMAT film (1–3 min exposure) or alkaline phosphatase (Promega, Madison, WI, USA) to assess each antibody's specificity. Quantitation of band intensity was performed using a computerized image analysis system (Northern Exposure, Empix Imaging, Mississauga, Ontario, Canada).

2.8. Implantation of intrastriatal cannula

Male Sprague-Dawley rats (300–350 g) were housed individually with free access to food and water throughout the study. All animals were kept in environmentally controlled rooms (25°C and 50% humidity) on a 12 h dark/light cycle. Following one week acclimatization, animals were anaesthetized with ketamine hydrochloride (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) prior to stereotaxic surgery. Guide cannulae (23-gauge hypodermic stainless steel) were implanted unilaterally into the right striatum at coordinates of 0.6 mm anterior and 3.5 mm lateral to bregma, and 3.8 mm ventral to the surface of the skull (Paxinos and Watson, 1986), with the mouthbar set at −3.5 mm. Surgical procedures were performed according to guidelines set by the Canadian Council on Animal Care and approved by the Animal Research Ethics Board (McMaster University, Hamilton, Ontario, Canada). At least one week after surgery, rats were administered a 0.5 μg dose of dopamine, norepinephrine, epinephrine, isopropylnorepinephrine, *N-n*-propylnorapomorphine and apomorphine, in a volume of 0.5 μl saline vehicle, over a 2 min period, once every day for 7 days. 8 μg of 6-hydroxydopamine was infused only once, in 4 μl of vehicle, over a 4 min period. At the end of all experiments the animals were killed, the striata removed on ice and stored at −70°C.

2.9. Administration of L-DOPA

Male Sprague-Dawley rats (300–350 g, $n = 10$) were gavaged orally with a 1 ml solution of saline containing 50 mg L-DOPA/11 mg carbidopa suspension for 7 days. After a 24 h washout period animals were killed, striata removed on ice and stored at −70°C. The control group received 1 ml of saline solution by oral gavage.

2.10. Reserpine treatment

Male Sprague-Dawley rats (300–350 g, $n = 6$) were injected with reserpine (1 mg/kg, s.c.) in a volume of 0.5 ml saline for 7 days at 24 h intervals. Control animals received only the vehicle solution. At the end of the experiment animals were killed, striata removed on ice and stored at −70°C.

3. Results

3.1. Identification of 47, 40 and 26 kDa proteins using iodinated ligand

The identification of CATNAPs, their regional brain distribution and their presence in all mammalian species tested have been previously reported by our laboratory (Mishra et al., 1992; Ross et al., 1993, 1995). Fig. 1 displays the typical labelling pattern of the 47, 40 and 26 kDa proteins by [^{125}I]DATN and [^{3}H]*N-n*-propylnorapomorphine in the presence and absence of 10 μM apomorphine in bovine striatum. Apomorphine was able to displace the three CATNAPs, when compared to the control lanes.

3.2. Protein purification

The concentrated protein samples, after SDS-PAGE and electroelution, were collected and characterized using a Biosil HPLC column. The HPLC chromatograms of the purified 47, 40 and 26 kDa proteins in Fig. 2 reveal the single peaks obtained, corresponding to 47, 40 and 26 kDa.

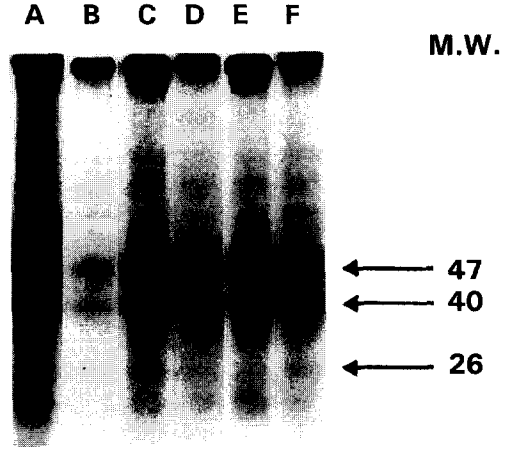

Fig. 1. Fluorography of catecholamine absorbing proteins (CATNAPs) from bovine striatum, labelled with [^{125}I]DATN and [^{3}H]*N-n*-propylnorapomorphine ([^{3}H]NPA). Lanes: A, 10 nM [^{125}I]DATN; B, 10 nM [^{125}I]DATN + 10 μM apomorphine; C, 1 nM [^{125}I]DATN; D, 1 nM [^{125}I]DATN + 10 μM apomorphine; E, 50 nM [^{3}H]NPA; F, 50 nM [^{3}H]NPA + 10 μM apomorphine.

3.3. Characterization and specificity of antibodies

The antibodies against the purified 47, 40 and 26 kDa protein preparations were raised in rabbits and the titre was determined by ELISA. The specificity of each antibody is demonstrated by Western blot analysis as shown in Fig. 3. Each antibody detects only its corresponding protein in both native striatal membranes and in the purified preparation. Furthermore, Fig. 4 demonstrates the localization of CATNAPs only in the rat striatum, but not in the rat liver, kidney, spleen, lung or heart. In addition, the localization of the 47 kDa protein in a wide variety of rat brain regions was examined (Fig. 5). By analysing the relative optical density of each band through computerized image analysis, it was determined that the rank order of regional distribution for the 47 kDa protein was as follows: striatum > cortex ≥ hippocampus ≥ nucleus accumbens > olfactory tubercle > hypothalamus ≥ medulla > cerebellum. These results are in agreement with the regional distribution of dopamine, epinephrine and norepinephrine in various brain regions (Glowinski and Iversen, 1966; Phyllis, 1970).

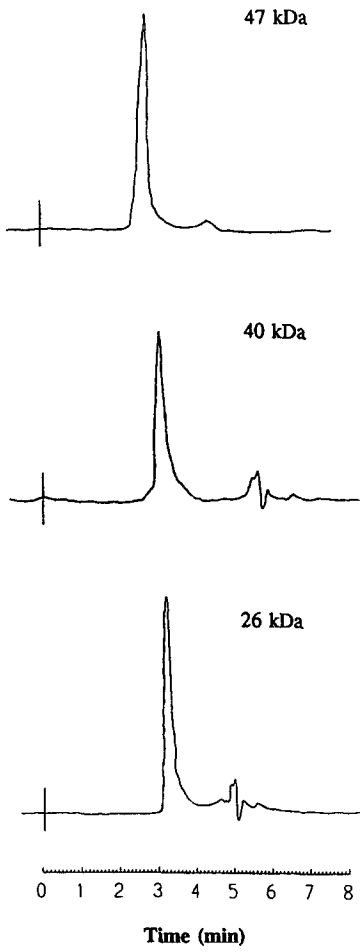

Fig. 2. HPLC profile of the three 47, 40 and 26 kDa proteins. Single peaks corresponding to each of the three CATNAPs were observed, following separation using the Biosil HPLC column.

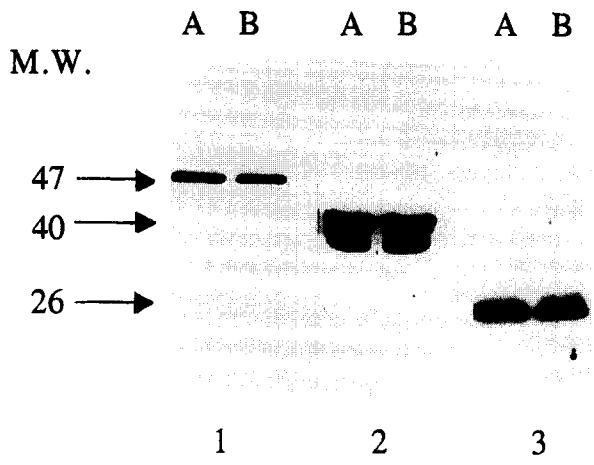

Fig. 3. Representative immunoblot of native (lane A) and purified (lane B) proteins. After separation on SDS-PAGE, the membrane and purified proteins were labelled with each antibody (lanes 1A and 1B, anti-47 kDA antibody; lanes 2A and 2B, anti-40 kDa antibody; lanes 3A and 3B, anti-26 kDa antibody) on three separate Western blots and detected by the ECL technique, as described in the Materials and methods section.

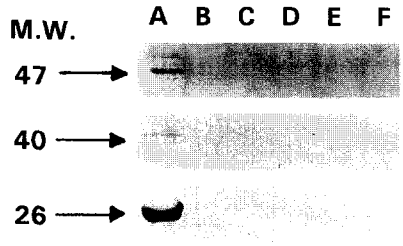

Fig. 4. Representative immunoblot showing the localization of the three CATNAPs, in various rat tissues. Using Western blot analysis and specific anti-CATNAP antibodies, CATNAPs were observed only in rat striatal membranes and not in any of the other tissues examined. Lanes: A, brain; B, liver; C, kidney; D, spleen; E, lung; F, heart.

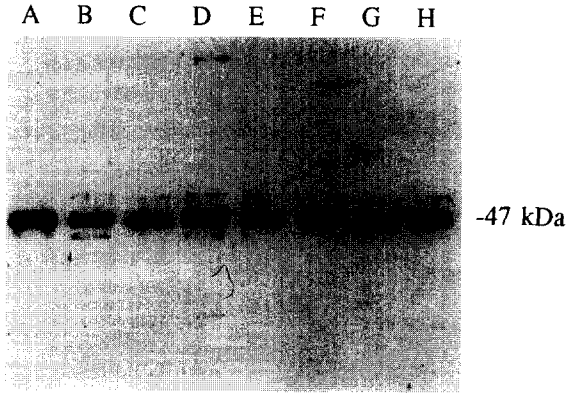

Fig. 5. Representative Western blot showing the regional distribution of the 47 kDa CATNAPs in the various rat brain regions. 20 μg of each membrane protein from the various brain regions was separated by SDS-PAGE and probed with anti-47 kDa antibody as described in the Materials and methods section. The films were analyzed using a computerized image analysis system and the intensity of the bands were taken as the concentration of protein present in each of the brain regions. Lanes: A, cortex; B, medulla; C, cerebellum; D, striatum; E, hippocampus; F, hypothalamus; G, nucleus accumbens; H, olfactory tubercle.

Table 1
Effect of chronic in vivo drug treatment on CATNAPs in the striatum

Treatment	Relative optical density units (% change) of CATNAPs		
	47 kDa	40 kDa	26 kDa
Control (100%)	560 ± 41	478 ± 29	350 ± 14
L-DOPA	895 ± 63 [a] (60↑)	760 ± 31 [a] (59↑)	590 ± 62 [a] (68↑)
Reserpine	264 ± 14 [a] (53↓)	305 ± 39 [a] (36↓)	198 ± 25 [b] (43↓)

Each value is an average of 5–6 separate experiments, ± S.E.M. A representative immunoblot is shown in Fig. 4. Values significantly different from controls are: [a] $P < 0.001$; [b] $P < 0.05$. The symbols ↑ or ↓ indicate an increase or decrease respectively, from control.

3.4. Effect of chronic in vivo administration of L-DOPA and reserpine on 47, 40 and 26 kDa protein levels

In order to determine the effect of catecholamine-inducing and catecholamine-depleting agents on CATNAP levels, animals were chronically treated with L-DOPA and reserpine. With the L-DOPA-treated animals, an increase of 60, 59 and 68% of the 47, 40 and 26 kDa proteins respectively was observed. However, the 47, 40 and 26 kDa CATNAPs decreased by 53, 36 and 43%, respectively, after reserpine treatment in rats (Table 1, Fig. 6).

3.5. Effect of intrastriatal administration of catecholamines on the 47, 40, 26 kDa protein levels

The effect of various dopaminergic and non-dopaminergic agents, administered via intrastriatal cannula on the three CATNAPs, is shown in the immunoblot in Fig. 7. The results obtained are shown in Table 2, where the relative optical densities were measured for each protein band and compared against the control values. *N-n*-Propylnorapomorphine-, apomorphine-, dopamine- and norepinephrine-treated animals showed an increase in the 47, 40 and 26 kDa proteins by significant amounts, while 6-hydroxydopamine-treated animals showed significant decreases in all three CATNAP levels.

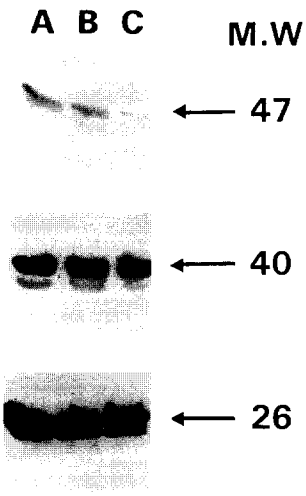

Fig. 6. Representative immunoblot showing the effect of in vivo treatment of rats with L-DOPA and reserpine on the three CATNAPs. Striatal membranes from the various drug-treated groups were prepared, probed with their respective anti-CATNAP antibodies and detected with ECL technique. Lanes: A, control; B, L-DOPA; C, reserpine.

4. Discussion

The production of free radicals as a result of catecholamine metabolism has been implicated as one of the major causes of central nervous system toxicity and neuronal injury. Many investigators have proposed that these toxic species play a key role in the pathogenesis of several neurodegenerative disease states (Olanow, 1992, 1993; Jenner, 1994). Free radicals formed by both enzymatic and non-enzymatic oxidation of catecholamines, specifically dopamine, have been implicated as major contributors in

Table 2
Effect of intrastriatal administration of dopaminergic and non-dopaminergic agents on CATNAPs in the striatum

Treatment	Relative optical density units (% change) of CATNAPs		
	47 kDa	40 kDa	26 kDa
Control (100%)	488 ± 37	588 ± 34	348 ± 32
Dopaminergic agents			
L-DOPA	720 ± 52 [a] (48↑)	1152 ± 106 [a] (96↑)	427 ± 24 [b] (22↑)
N-n-Propylnorapomorphine	690 ± 42 [a] (41↑)	1104 ± 129 [a] (88↑)	452 ± 37 [b] (30↑)
Apomorphine	680 ± 48 [a] (40↑)	1125 ± 137 [a] (91↑)	438 ± 29 [b] (26↑)
Dopamine	710 ± 69 [a] (46↑)	1205 ± 168 [a] (105↑)	472 ± 58 [b] (36↑)
6-Hydroxydopamine	187 ± 22 [a] (62↓)	228 ± 29 [a] (62↓)	204 ± 22 [a] (42↓)
Non-dopaminergic agents			
Epinephrine	527 ± 51 (8↑)	597 ± 47 (–)	457 ± 36 [b] (31↑)
Norepinephrine	576 ± 39 (18↑)	760 ± 62 [b] (29↑)	468 ± 42 [b] (34↑)
Isopropylnorepinephrine	484 ± 32 (–)	576 ± 47 (–)	378 ± 29 (9↑)

Each value is an average of 5–6 separate experiments, ± S.E.M. Representative immunoblots are shown in Fig. 5 (dopaminergic agents) and Fig. 6 (non-dopaminergic agents). Values significantly different from controls are: [a] $P < 0.01$; [b] $P < 0.05$. [c] L-DOPA was administered orally. The symbols ↑ or ↓ indicate an increase or decrease respectively, from control.

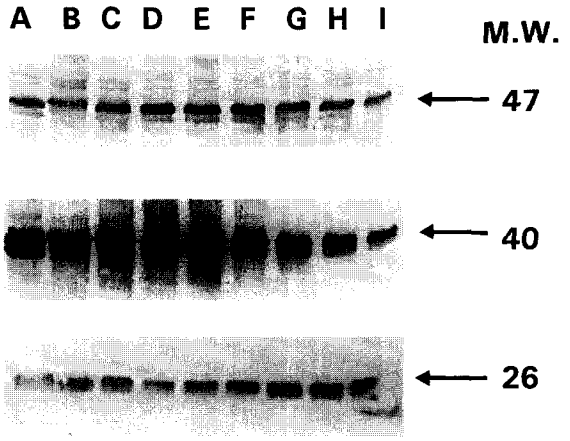

Fig. 7. Representative immunoblot showing the effect of L-DOPA and intrastriatal injections of catecholamines on CATNAPs in rats. The membranes from various drug-treated tissues were prepared and the level of CATNAPs were detected by Western blot using anti-CATNAP antibodies. Lanes: A, L-DOPA; B, control; C, NPA; D, apomorphine; E, dopamine; F, norepinephrine; G, epinephrine; H, isopropylnorepinephrine; I, 6-hydroxydopamine.

the cell death associated with neurodegenerative disease. The membrane bound CATNAPs characterized in this report are a family of membrane bound proteins found exclusively in the brain. These proteins may have an ability to react with the free radicals formed by the oxidative mechanism of dopamine degradation and provide protection against further neuronal degeneration or cell death.

This report outlines in detail the identification, pharmacological characterization and modulation of brain specific CATNAPs, using polyclonal antibodies generated against the 47, 40 and 26 kDa proteins. From the representative chromatograms in Fig. 2, the presence of a single peak is evidence for the high purity of each of the proteins. Furthermore, Western blot analysis of the three purified antibodies performed in striatal membranes revealed a single band in striatal but not peripheral tissues, which confirms the specificity of these antibodies and demonstrates the exclusive localization of these proteins in the brain. In addition, these proteins (data shown for 47 kDa) display differential regional distribution that is consistent with the approximate regional brain concentrations of catecholamines (Fig. 5) (Glowinski and Iversen, 1966; Phyllis, 1970) as determined by the computerized image analysis of band intensities.

L-DOPA is known to increase dopamine levels in the striatum of intact animals (Abercrombie et al., 1990). In the present study, 50 mg/kg of L-DOPA administered to intact animals caused a significant increase of all of the three CATNAP levels. This result is consistent with our previous findings, where radiolabelled DATN was used (Mishra et al., 1992). We propose that in intact animals most of the dopamine formed in response to the administration of low doses of L-DOPA is adequately stored in dopaminergic neurons. However, if higher doses of L-DOPA are given to the intact animal, an excess of dopamine is formed and the dopamine uptake sites become saturated. This excess dopamine spills out from the dopaminergic and non-dopaminergic neurons and produces an elevated dopamine concentration in extracellular fluid, where it undergoes catabolic processes that generate free radicals. Importantly, our results have demonstrated an increase of CATNAPs in response to the high L-DOPA dose administered. It is hypothesized that the excess of dopamine is rapidly absorbed by the CATNAPs before the dopamine oxidase catalyzes the formation of reactive metabolites (free radicals).

In addition to L-DOPA treatment, we have administered catecholamines and their analogues directly into the striatum, via implanted cannulae. These experiments were performed in order to ascertain whether the increase of CATNAPs could be brought about by increasing the concentration of monoamines directly. The results shown in Fig. 7 and Table 2 clearly indicate that the increased levels of dopamine and related catecholamines produced an effect similar to that obtained with L-DOPA treatment. In animals treated with the catecholamine depleting agents, reserpine and 6-hydroxydopamine (Tables 1 and 2), decreased levels of CATNAPs were observed. Therefore, these results suggest that when the concentration of catecholamines in the synaptic area is increased above a certain limit, the levels of CATNAPs also increase, in an attempt to maintain cellular homeostasis. These results support the hypothesis that the CATNAPs play an important cytoprotective role, by preventing the damage caused by free radicals generated by the oxidative stress derived from increased catecholamine metabolism. Whether similar changes occur in neurodegenerative disorders such as Parkinson's disease is not yet known.

In conclusion, we have provided immunological and pharmacological evidence for the functional role of catecholamine absorbing proteins in neuronal processes that occur in the brain. This work also provides a framework for further investigation of the role of such proteins in brain function and the pathogenesis of various neurodegenerative disorders.

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