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SELECTIVE DECREASE IN THE AFFINITY OF D_2 DOPAMINE RECEPTOR FOR AGONIST INDUCED BY ISLET-ACTIVATING PROTEIN, PERTUSSIS TOXIN, ASSOCIATED WITH ADP-RIBOSYLATION OF THE SPECIFIC MEMBRANE PROTEIN OF BOVINE STRIATUM

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SUMMARY : When the crude synaptic membrane preparations from bovine striatum were treated with islet-activating protein (IAP), one of the pertussis toxins, a protein with a molecular weight of about 40,000 was ADP-ribosylated. In parallel with this ADP-ribosylation, there was a decrease in D_2 dopamine receptor affinity for agonist, while the affinity for antagonist remained unaltered. Addition of GTP to nontreated membranes also resulted in a decrease in the affinity of D_2 receptor for agonist, and there was no further reduction of affinity for agonist with addition of GTP to the IAP-treated membranes. As IAP specifically acts on the guanine nucleotide regulatory protein which mediates the inhibition of adenylate cyclase activity (Ni), our findings indicate a possible molecular interaction between the brain D_2 dopamine receptor and Ni.

Brain D_2 dopamine receptor is considered to be responsible for the behavioral and clinical effects of neuroleptics (1). Pharmacological and biochemical studies of striatal D_2 dopamine receptor revealed that stimulation of this type of receptor is followed by a reduction in cyclic AMP efflux from slices of rat neostriatum, probably the result of inhibition of adenylate cyclase (2), and that the binding affinity of the receptor is regulated by guanine nucleotide (3,4). The stimulation and inhibition of adenylate cyclases were proposed to be mediated by distinct guanine nucleotide regulatory proteins named Ns and Ni, respectively (5-10). All these findings led to the speculation of a possible molecular interaction between the brain D_2 dopamine receptor and the guanine nucleotide regulatory protein which mediates the inhibition of adenylate cyclase activity (Ni). IAP, one of the pertussis toxins, reportedly blocks hormone and neurotransmitter receptor-mediated inhibition of adenylate cyclase activity and modulates the binding affinities of these

ABBREVIATIONS : IAP, islet-activating protein; SDS, sodium dodecyl sulfate.

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receptors, presumably as a result of ADP-ribosylation of one of the subunits of Ni (6-10). We report here that IAP modulates the binding affinity of the bovine striatal D_2 dopamine receptor, thus a molecular interaction between brain D_2 dopamine receptor and Ni has to be considered.

MATERIALS AND METHODS

Materials : IAP purified from the 3-day culture supernatant of *B. pertussis* cells (Tohama strain, Phase I) according to the procedure described elsewhere (11) was generously provided by Dr. M. Yajima (Research Laboratories of Kakenyaku Kako Co., Shiga, Japan). The stock solution was prepared by dissolving 1 mg of IAP in 1 ml of the vehicle consisting of 0.1 M potassium phosphate buffer (pH 7.0) and 2 M urea, and storing at 4°C until use. The vehicle alone was used as control. Sulpiride (Fujisawa, Japan), (-)- and (+)-butaclamol (Ayerst, Canada), ketanserin (Kyowa Hakko, Japan) and haloperidol (Yoshitomi, Japan) were gifts from the respective companies. [^{3}H]-spiperone (31.7 Ci/mmol) and [^{32}P]NAD (52 Ci/mmol) were purchased from New England Nuclear. ATP, GTP, NAD, thymidine, dithiothreitol, dopamine hydrochloride and apomorphine hydrochloride were purchased from Sigma Chemical Co.

Membrane preparation : Crude synaptic membrane preparations were prepared as described previously (4). In brief, striata dissected from bovine brain were homogenized in 20 volumes of ice cold 50 mM-Tris-HCl buffer, pH 7.4, with a Brinkmann Polytron PT-10 (setting 7, 20 s). The homogenate was centrifuged (50,000 g, 10 min) three times, with resuspension of the intermediate pellet in fresh buffer. The final pellet was suspended in 2 volumes of 25 mM-Tris-HCl buffer, pH 7.4, containing 2.5 mM $MgCl_2$, and stored at -70°C until use.

Treatment of membranes with IAP : The membrane preparation (15-20 mg of protein) was incubated with 125 µg of preactivated IAP for 15 min at 37°C in 5 ml of 25 mM-Tris-HCl buffer, pH 7.4, containing 2.5 mM $MgCl_2$, 1 mM ATP, 0.2 mM GTP, 10 mM thymidine, 5 mM dithiothreitol and 1 mM NAD. Incubation was terminated by cooling the reaction tube followed by centrifugation (10,000 g, 10 min) at 4°C. The pellet was washed three times with ice cold 50 mM-Tris-HCl buffer, pH 7.4, by repeating dilution and centrifugation. For radiolabeling, 10 µM [^{32}P]NAD (52 Ci/mmol) was used instead of nonradioactive NAD. IAP was preactivated by incubation in 20 mM dithiothreitol for 15 min at 37°C.

[^{3}H]spiperone binding : Membrane preparations (0.3-0.6 mg of protein) were incubated with [^{3}H]spiperone in 0.6 ml of 50 mM-Tris-HCl buffer, pH 7.4, containing 120 mM NaCl and indicated concentrations of drugs. Tubes were incubated for 30 min at 25°C and the incubations were terminated by vacuum filtration of the membrane through Whatman GF/B filters followed by three washings with 5 ml of ice cold 50 mM-Tris-HCl buffer, pH 7.4. Radioactivity in the filters was counted in a toluene-base scintillator by LS-7000 Beckman scintillation spectrometer at 48 % efficiency. Stereospecific binding was determined as the difference in the binding obtained with incubation in the presence of 1 µM (+)-butaclamol and 1µM (-)-butaclamol. D_2 receptor specific binding was defined as the binding of [^{3}H]spiperone that occurred in the presence of 0.1 µM ketanserin (to occlude S_2 serotonergic sites) but that was displaceable by 1 µM (+)-butaclamol. Protein was assayed by the method of Lowry et al. (12).

Polyacrylamide gel electrophoresis and autoradiography : The discontinuous buffered SDS-polyacrylamide slab gel electrophoresis was used to analyse the labeled membrane preparation, as described previously (13). In brief, the crude synaptic membrane preparations recovered from the incubation with IAP and [^{32}P]NAD were reduced with 5 % β-mercaptoethanol in 62.5 mM-Tris-HCl

buffer, pH 6.8, containing 2 % SDS, 10 % glycerol and 0.001 % bromphenol blue by boiling for 5 min before electrophoresis on 10.5 % gel. After staining with Coomassie blue and destaining, the gel slab was dried for autoradiography on Kodak X-Omat R film. The molecular weights of bands of the gels were estimated by the method of Weber and Osborn (14).

RESULTS AND DISCUSSION

ADP-ribosylation of membrane protein by IAP : Fig. 1 shows the radiolabeled pattern of membrane protein after incubation of membrane preparations from bovine frontal cortex and striatum with [^{32}P]NAD in the presence or absence of IAP. IAP treatment of membrane preparations from bovine frontal cortex and striatum resulted in labeling of a protein with a molecular weight about 40,000. These results are consistent findings obtained using C6 glioma cells (8) or *cyc*$^-$ S49 cells (10). This seems to be the first evidence that mammalian brain membranes also contain a pertussis toxin substrate, probably one of the subunits of Ni (8,10).

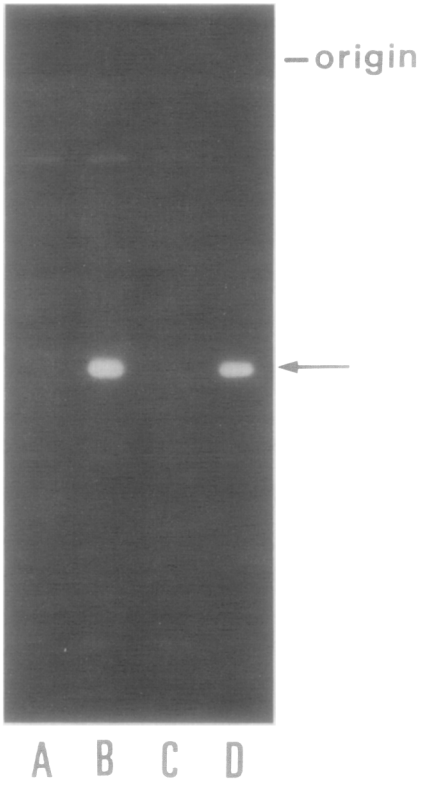

Figure 1. Polyacrylamide gel analysis of bovine brain membranes from frontal cortex (A,B) and striatum (C,D) labeled with [^{32}P]NAD in the presence (B,D) or absence (A,C) of IAP. Membranes were incubated with [^{32}P]NAD and then electrophoresed, as described in the text. The arrow indicates the 40,000 dalton band.

TABLE 1

Kinetic parameters for the D_2 dopamine receptor specific binding of [^{3}H]-spiperone to IAP-treated and nontreated bovine striatal membranes

	B_{max} (fmol/mg protein)	K_D (nM)
Control	200 ± 10	0.32 ± 0.02
IAP-treated	210 ± 20	0.34 ± 0.03

Crude synaptic membrane preparations that had been treated with and without 25 μg/ml of IAP were incubated with various concentrations of [^{3}H]-spiperone in the presence of 0.1 μM of ketanserin, as described in the text. The maximum binding (B_{max}) and dissociation constant (K_D) for the binding were estimated from the Scatchard plot of the specific binding, determined as described in the text. Each value represents mean ± S.E.M. of three independent experiments.

[^{3}H]spiperone binding to IAP-treated and nontreated membranes : D_2 receptor specific binding of [^{3}H]spiperone to IAP-treated and nontreated bovine striatal membranes was measured in the presence of increasing concentrations of [^{3}H]-spiperone. Scatchard analysis of both membrane preparations gave a linear relationship indicating a single class of specific binding sites (data not shown). The numbers of binding sites (B_{max}) as well as the dissociation constants (K_D) for binding were then estimated, as shown in Table 1. There was no difference in these kinetic parameters between IAP-treated and nontreated membranes, indicating that neither the density of D_2 receptors nor the affinity for [^{3}H]spiperone was affected by IAP treatment.

Displacement of [^{3}H]spiperone binding to IAP-treated and nontreated membranes by dopamine agonists and antagonists : The displacement of [^{3}H]spiperone binding to IAP-treated and nontreated bovine striatal membranes by dopamine agonists as well as antagonists, in the presence or absence of 100 μM GTP, is shown in Table 2. With the IAP treatment, IC_{50} concentrations for dopamine (40 μM) and apomorphine (2.1 μM) with nontreated membranes were significantly increased to 120 μM and 4.8 μM, respectively, while those for sulpiride, haloperidol and (+)-butaclamol were not significantly changed. Addition of GTP to nontreated membranes also resulted in a selective decrease in the affinity of the D_2 receptor for agonists, as described in previous reports

TABLE 2

IC_{50} concentrations of dopamine agonists and antagonists for stereospecific [^{3}H]spiperone binding to IAP-treated and nontreated bovine striatal membranes in the absence or presence of 100 μM GTP

	IC_{50} concentration (μM)			
	Control	IAP	GTP	IAP + GTP
Dopamine	40 ± 3	120 ± 10*	120 ± 10*	130 ± 10*
Apomorphine	2.1 ± 0.2	4.8 ± 0.4*	5.0 ± 0.4*	5.0 ± 0.4*
Sulpiride	10 ± 2	8.8 ± 1.2	9.0 ± 1.2	8.5 ± 1.5
Haloperidol	0.060 ± 0.004	0.071 ± 0.008	0.066 ± 0.008	0.058 ± 0.007
(+)-Butaclamol	0.016 ± 0.002	0.018 ± 0.003	0.016 ± 0.003	0.016 ± 0.002

Crude synaptic membrane preparations that had been treated with or without 25 μg/ml of IAP were incubated with 1 nM [^{3}H]spiperone in the presence of various concentrations of indicated drugs, in the presence or absence of 100 μM GTP, as described in the text. Stereospecific binding of [^{3}H]spiperone was defined as described in the text. Each value represents the mean ± S.E.M. of three independent experiments. Significance : * $P < 0.05$ (versus control value)

(3,4). IC_{50} concentrations for dopamine and apomorphine were 120 μM and 5.0 μM in the presence of 100 μM GTP, respectively. However, there was no further reduction of affinity for agonists upon addition of GTP to the IAP-treated membranes. Thus, the guanine nucleotide regulatory protein (Ni) does not seem to be coupled to the D_2 dopamine receptor in IAP-treated membranes. On the other hand, there was no changes in the affinity of D_2 receptor for agonists or antagonists with the cholera toxin treatment, and IAP-induced modulation of the D_2 dopamine receptor was not observed when the incubation mixture for IAP treatment did not contain NAD (data not shown). Our results obtained here (that IAP treatment of bovine brain membranes induced a lowering in the affinity of D_2 receptors for agonists, a loss of sensitivity to GTP in agonist binding and ADP-ribosylation of the specific membrane protein) suggest that ADP-ribosylation of one of the subunits of Ni leads to the conformational change of D_2 receptor protein, through the molecular interaction between these two proteins. These results together with the finding that D_2 receptor stimulation induced a reduction in cyclic AMP efflux from striatal slices (2) suggest bidirectional coupling of Ni to

adenylate cyclase and D_2 receptor as well as α_2 adrenergic, cholinergic muscarinic and opiate receptors (9).

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