Vol. 167, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1990 Pages 211-215

GUANINE NUCLEOTIDES REGULATE THE EFFECT OF SUBSTANCE P ON STRIATAL ADENYLATE CYCLASE OF THE RAT

A. MOSER

Dept of Neurology, Med University, Ratzeburger Allee 160, D-2400 Lübeck, F.R.G.

Received January 4, 1990

Substance P was incubated in an adenylate cyclase assay of a particulate fraction of caudate-putamen tissue of the rat in order to examine the effect of the peptide on D-1 receptor coupled adenylate cyclase in vitro. Substance P did not influence basal adenylate cyclase activity or the stimulation of the enzyme by dopamine. No influence of substance P was seen on the effects of calcium and magnesium chloride as a cofactor of adenylate cyclase. Also the inhibition of adenylate cyclase activity by the dopamine antagonist fluphenazine was not influenced by substance P. However, substance P was able to enhance cyclic AMP formation in the presence of guanosine-imido-diphosphate (Gpp(NH)p), whereas the stimulatory effect of guanosine-triphosphate (GTP) was inhibited by substance P. In our study we suggest that substance P interacts with the guanine nucleotide regulatory subunit without directly affecting D-1 dopamine receptors in the caudate-putamen of the rat.

Substance P is an undecapeptide widely distributed in the mammalian central nervous system (7, 14). High amounts are present in the pericarya and axons of neurons of the striatonigral pathway, where it may act as a neurotransmitter (11, 12, 18). Substance P was found to excite nigral neurons (2, 6). In Huntington's and Parkinson's diseases substance P levels have been found decreased in the striatum and the substantia nigra (8, 23). However, evidence for or against a modulator function of substance P on striatal dopamine receptors has not yet been reported. In order to clarify a potential interaction between substance P and D-1 receptors in this brain area we used a pellet preparation of rat caudate-putamen to measure dopamine sensitive adenylate cyclase activity and the effects of substance P thereupon.

MATERIAL AND METHODS

Female wistar rats of 150-200 g body weight were used. Rats were decapitated and caudate-putamen were dissected on ice. For each

0006-291X/90 $1.50

pellet preparation identical CP (caudate-putamen) from two rats were combined to reduce inter-individual differences.
The tissues were homogenized (10 strokes) in an ice cold calcium-free solution of 10 mM TRIS-HCl and 4 mM EDTA, pH 7.6 (previously perfused with 95% oxygen, 5% carboxygen), centrifuged at 1500 x g and resuspended and recentrifuged three times at 0 deg Celcius. The resulting particle suspension was used as a source of enzyme immediately after preparation.
Protein was determined according to Bensadoun and Weinstein (1). According to our previous studies (17) the incubation medium was composed of approximately 10 µg membrane protein, 0.4 mM magnesium chloride, 0.1 mM papaverine 0.1 mM 5-adenylyl-imido-diphosphate, approximately 10 µCi (32-P)-ATP (1000,000 cpm/tube), 10 µg/ml adenosine desaminase, 0.1 M glycylglycine buffer, pH 7.4 and the indicated substances. The assay was linear with protein concentrations up to 100 µg and with incubation times up to 30 min. The standard incubation time was 8 min. at 30 deg Celcius. The reaction was determined by the addition of a solution of 10 mM ATP, 10 mM cyclic AMP, sodium dodecylsulfate 2% in 50 mM TRIS-HCl, pH 7.4. The formation of (32-P)-cyclic AMP was measured by separation of the reaction product through sequential chromatography on dowex 50 cation exchanger and on neural alumina. Within each experiment adenylate cyclase measurements were replicated 3 times for each experimental condition. Values were expressed in pmoles cyclic AMP/min/mg protein ± standard deviation (SD). For statistical analysis Student's t-test was employed.

RESULTS

In the presence of substance P (0.01 - 10 µM) no change in the basal adenylate cyclase was observed (data not shown). Dopamine enhanced the formation of cyclic AMP with a maximal increase over basal activity occurring at 0.1 mM (table 1). Stimulation of adenylate cyclase produced by a submaximal or maximal concentration of dopamine was not influenced by substance P.

Table 1

Effects of calcium and magnesium chloride, dopamine, fluphenazine, guanylylimido-diphosphate, and guanosine-triphosphate in the absence and presence of substance P (1 µM) on the adenylate cyclase activity in pmoles cyclic AMP/min/mg protein ± SD

	- substance P	+ substance P
control	6.0 ± 0.5	7.2 ± 0.8
calcium (2 mM)	0.6 ± 0.4 *	0.6 ± 0.2
magnesium (4 mM)	16.7 ± 0.5 *	17.4 ± 0.1
dopamine (0.1 mM)	14.0 ± 0.5 *	14.1 ± 0.8
dopamine + fluphenazine (10 µM)	5.4 ± 0.5	5.7 ± 0.4
Gpp(NH)p (0.1 mM)	42,3 ± 3 *	61.2 ± 3 **
GTP (0.1 mM)	13.5 ± 0.2 *	7.8 ± 0.5**

* $p < 0.01$ significant compared to control
** $p < 0.01$ significant compared to - substance P

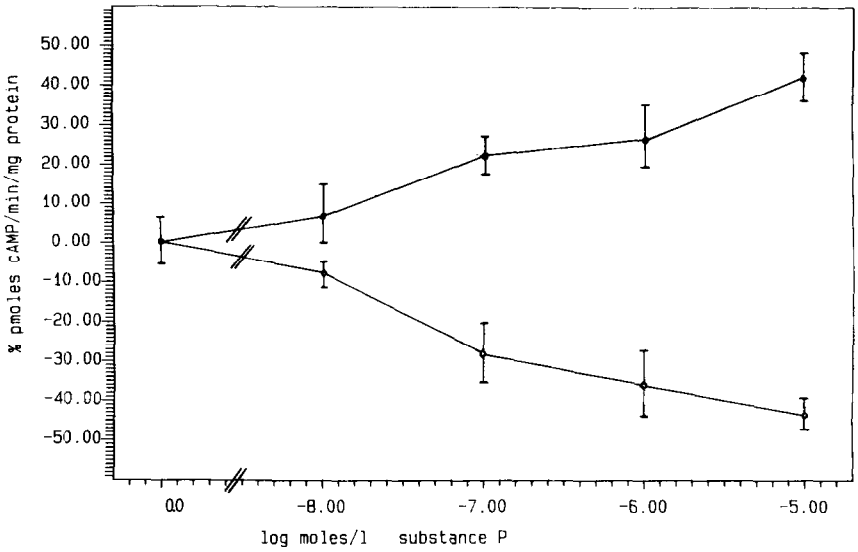

Fig.1
Effect of substance P in the presence of guanosine-triphosphate (○) or in the presence of guanylylimido-diphosphate (●) in percent of basal adenylate cyclase activity ± SD.

Fluphenazine was able to inhibit the dopamine-stimulated adenylate cyclase activity. The inhibition induced by fluphenazine (10 µM) was not influenced by substance P (table 1). Adenylate cyclase actvity of CP preparations was inhibited markedly by addition of calcium ions (3). On the other hand magnesium ions considerably enhanced the formation of cyclic AMP. The effects of both calcium chloride over a range of concentrations from 0.2 to 2 mM and magnesium chloride from 4 µM to 4 mM were not affected by addition of substance P 1 µM (table 1). When the guanosine nucleotide Gpp(NH)p was present in the incubation medium substance P (1 µM) stimulated the enzyme activity to approximately 130 % when compared to control (table 1). Substance P was most effective in a concentration of 10 µM (fig. 1). In contrast to this, after employment of guanosine-triphosphate (GTP) substance P inhibited adenylate cyclase activity in a dose dependent manner with a apparent v-max that was 58 % of the GTP (10 µM) induced response (table 1 and fig. 1).

DISCUSSION

While there is general agreement that D-2 receptors in basal ganglia are directly involved in extrapyramidal motor control, the physiological functions of D-1 receptors and D-1 receptor

coupled adenylate cyclase are still unknown (5). In the caudate-putamen D-1 receptors appear to be restricted to striatal intrinsic neurons (13, 16). Substance P is localized in spiny I or II efferent neurons of the striatum (9).

In our experiments substance P did not influence the dopamine stimulation of adenylate cyclase in a particulate preparation containing synaptosomes of the rat caudate-putamen (17). Also the inhibition of the dopamine-activated adenylate cyclase by fluphenazine was not influenced by substance P. Thus, an effect of substance P on D-1 dopamine receptor system seems unlikely.

Cheramy et al (2) have reported that microionto-phoretical application of substance P in the substantia nigra caused an enhancement of dopamine concentrations in the striatum, while injection of substance P into the striatum inhibited both the release and the uptake of dopamine (21). However, the interaction at the molecular level of substance P-ergic neurons with dopaminergic afferents is largely unknown.

The guanine nucleotide analogues are essential cofactors for hormonal stimulation of adenylate cyclase (10, 19, 20). The guanine nucleotide sensitive subunit of the enzyme presents a multicomponent regulatory complex including inhibitory and excitatory binding sites (4), at which hormones or neuromodulators might interact (20). Sunyer et al (22) found that G-i is capable of hydrolyzing GTP where the natural effector GTP presented the highest affinity of the GTP-ase system in comparison to other guanosine nucleotide analogues like Gpp(NH)p. Since in our preparation substance P was able to modulate striatal adenylate cyclase activity if guanine nucleotides were present the effect of substance P seems actually to be associated with guanine nucleotide regulatory subunit showing both a stimulatory or inhibitory effect according to which guanine nucleotide is present. In agreement with Macdonald and Boyd (15), who have found, that in peripheral tissues the effect of the binding of substance P to receptors is mediated by a G-protein, that is required for high affinity binding, we could demonstrate an interaction between substance P and the guanine nucleotide regulatory subunit of an adenylate cyclase of the rat caudate-putamen.

REFERENCES

1. Bensadoun A and Weinstein D (1976) Anal Biochem 70, 241-250.

2. Cheramy A; Nieoullon A, Michelot R, Glowinski J (1977) Neurosci Lett 4, 105-109.

3. Clement-Cormier YC, Parrish RG, Petzold GL, Kebabian JW, Grenngard P (1975) J Neurochem 25, 143-149.

4. Codina J, Hildebrandt JD, Sunyer T, Sekura RD, Manclark CR, Iyengar R, Birnbaumer L (1984) Adv Cyclic Nucl Res 17, 111-125.

5. Creese I (1982) Trends Neurosci 40-43.

6. Davies J and Dray A (1976) Brain Res 107, 623-627.

7. Eckernäs SA, Aquilonius SM, Lundquist G, Roose BE (1978) Acta Neurol Scand 57, 225-226.

8. Gale JS, Bird ED, Spokes EG, Iversen LL, Jessel T (1978) J Neurochem 30, 633-634.

9. Groves PM (1983) Brain Res Rev 5, 109-132.

10. Hildebrandt JD, Sekura RD, Codina J, Iyengar R, Manclark CR, Birnbaumer L (1983) Nature 302, 707-709.

11. Hong JS, Hang HYT, Costa E (1977) Brain Res 122, 541-544.

12. Kanazawa I, Emson PC, Cuello AC (1977) Brain Res 119, 447-453.

13. Kebabian JW and Calne B (1979) Nature 277, 96-99.

14. Lembeck F, Mayer N, Schindler G (1977) Naunyn Schmiedeberg's Arch Pharmacl 301,17-22.

15. Macdonald SG and Boyd ND (1989) J Neurochem 53, 264-272.

16. McGonigle P, Huff RM, Molinoff B (1984) Ann NY Acad Sci 430, 77-90.

17. Moser A, Reavill C, Jenner P, Marsden CD, Cramer H (1986) Exp Brain Res 62, 567-571.

18. Mroz EA, Brownstein MJ, Leeman SE (1977) Brain Res 125, 305-311.

19. Normura Y, Arima T, Swgawa T (1987) Int J Devl Neurosci 5, No 3, 271-279.

20. Rodbell M, Lad PM, Nielson TB, Cooper DMF, Schlegel W, Preston MS, Londos C, Kempner ES (1981) Adv Cyclic Nucl Res 14, 3-14.

21. Silbergeld EK and Walters JR (1979) Neurosci Lett 12, 119-126.

22. Sunyer T, Codina J, Birnbaumer L (1984) J Biol Chem 259, No 24, 15447-15451.

23. Tenovou O, Rinne UK, Viljanen MK (1984) Brain Res 303, 114-116.