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Effects of several ergosines on adenylate cyclase activity in synaptosomal membranes of the bovine caudate nucleus

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The effects of several ergosines and different dopamine agonists and antagonists on the activity of dopamine-sensitive adenylate cyclase in synaptosomal membranes of the bovine caudate nucleus were comparatively studied. Among ergot alkaloid derivatives used, ergosinine was the most active in stimulating adenylate cyclase activity. Ergosine, bromoergosine, dihydroergosine, dihydroergocryptine and lisuride also stimulated this enzyme. Dihydroergosinine, bromodihydroergosine and bromoergocryptine did not affect adenylate cyclase activity. Saccharino derivatives of both ergosine and ergosinine were inactive. When used in higher concentrations, ergosine, ergosinine, dihydroergocryptine and lisuride inhibited dopamine-stimulated adenylate cyclase whereas other ergot alkaloid derivatives examined did not. If the extent of dopamine-sensitive adenylate cyclase stimulation is considered as a measure of dopaminergic activity, examination of the structure/dopaminergic activity relationship showed that modifications of ergot alkaloid molecules such as isomerization in position 8, hydrogenation of $\Delta^{9(10)}$-double bond, or introduction of bromine into position 2 of the molecule, lead to a significant decrease of stimulatory effects of adenylate cyclase. Introduction of a saccharino group into position 2 of the molecule caused a total loss of stimulatory activity of both ergosine and ergosinine, probably because of the size of the saccharino residue.

Numerous publications have demonstrated the dopaminergic activity of some ergot alkaloids (Johnson et al 1976; Cannon et al 1981; Euvrard et al 1981; Holohean et al 1982; Lataste 1984) and have led to the introduction of these compounds in the therapy of disorders related to the dysfunction of dopaminergic systems (Berde 1978; Stadler 1980). However, ergot alkaloids from the ergosine series have been neglected. Recent pharmacodynamic studies (Djordjević, personal communication) have suggested that ergot alkaloids from ergosine series have dopaminergic activity. This has prompted us to investigate the effects of these substances on the activity of dopamine-sensitive adenylate cyclase (EC 4.6.1.1) in synaptosomal membranes of the bovine caudate nucleus. Increased synthesis of cAMP is one of the earliest events after dopamine treatment (Seeman 1980) and changes in adenylate cyclase activity may be used to estimate the dopaminergic activity of dopamine agonists (Clement-Cormier et al 1979; Seiler & Markstein 1982). The literature concerning the effects of ergot alkaloids on dopamine-dependent adenylate cyclase, provides an unclear picture of the ergot alkaloid/adenylate cyclase activity relationship (von Hungen et al 1975; Trabucchi et al 1976; Schmidt & Hill 1977; Fuxe et al 1978; Azuma & Oshino 1980) because of different experimental approaches. Our results demonstrate prominent stimulatory effects of several ergot alkaloids from ergosine series on adenylate cyclase activity in synaptosomal membranes of the bovine caudate nucleus.

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Material and methods

Synaptosomal membrane preparation. Nuclei caudata were dissected from bovine brains obtained from a local abattoir 2 h after death. Synaptosomal membranes were prepared by the method of Nishikori et al (1980) for preparation of the M_1 fraction. The final pellet was resuspended in 80 mM Tris HCl, 0·6 mM Na_4EDTA, pH 7·4 to produce a protein concentration of 10 mg ml^{-1} and divided into 4·0 ml aliquots, which were frozen in liquid nitrogen and kept at −20 °C.

The protein concentration was determined according to Lowry et al (1951) using a bovine serum albumin as a standard.

Determination of adenylate cyclase activity. The activity of adenylate cyclase was determined as recommended by Clement-Cormier et al (1975), but instead of 0·5 mM ATP as in the original procedure, 2 mM ATP was used. Standard incubation mixtures contained in a final volume of 0·5 ml: 400 μl of the buffer (10 mM theophylline, 80 mM Tris HCl, 0·6 mM Na_4EDTA, 8 mM $MgSO_4$, 0·02% ascorbic acid, pH 7·4), 50 μl of various amounts of the drugs examined and 50 μl of the membrane suspension (final concentration of protein 100 μg ml^{-1}). After 30 min at 4 °C, incubation mixtures were transferred to 30 °C, and 60 s later the reaction was initiated by the addition of 50 μl of 20 mM ATP and 0·5 mM GTP in the previous buffer. In some experiments, GTP was omitted. After 5 min, the reaction was stopped by transferring the mixture to a boiling water bath for 3 min. After centrifugation (15 min, 800*g*, Sorwall SS_1 centrifuge), 50 μl aliquots of the supernatants were used for determination of cAMP content according to Brown et al (1971), which was taken as a direct measure of adenylate cyclase activity. Under these experimental conditions, enzymatic activity was directly proportional to the duration of incubation and protein concentration in the membrane preparations.

Radioactivities were measured in a Packard Tri-Carb spectrometer at an efficiency of 45%, using scintillation liquid after Bray (1960).

Chemicals. [^{3}H]Cyclic adenosine-3′,5′-monophosphate (spec. act. 34 Ci $mmol^{-1}$) was from NEN, Boston, MA, USA.

The following drugs were kindly donated: (+)- and (−)-butaclamol (Ayerst Labs., Montreal, Canada); apomorphine, bromoergocryptine (BrEC) and dihydroergocryptine (DHEC; Sandoz Co., Basel, Switz.); lisuride (Dr G. N. Woodruff, Merck, Sharp and Dohme, Neurosci. Res. Ctr., Hoddesdon Herts, UK); haloperidol (Janssen Pharmaceutica, Beerse, Belgium); chlorpromazine (E. R. Squibb and Sons, Princeton, USA); metoclopramide (Delagrange, Paris, France).

Ergosine, ergosinine, dihydroergosine (DHESN), bromoergosine (BrESN), dihydrobromoergosine (BrDHESN), saccharinoergosine (SacchESN) and saccharinoergosinine (SacchESNN) methanesulphonates were products of 'LEK' Pharmaceutical and Chemical industry, Ljubljana, Yugoslavia.

All other chemicals were analytical grade from Sigma.

Results

Stimulation of adenylate cyclase activity by several commonly used dopaminergic ergot alkaloids and different ergosines is shown in Fig. 1 and values for the activation constant (K_{act}) and maximal relative enzyme activities are listed in Table 1.

It is obvious that ergosinine was the best stimulant of adenylate cyclase. Ergosine, BrESN, DHESN, DHEC and lisuride also had stimulant activity, while DHESNN, BrDHESN and BrEC did not affect the activity of the enzyme. Saccharino derivatives of both ESN and ESNN were inactive.

Table 1. Effects of dopamine agonists, dopaminergic ergot alkaloids and some ergosines on adenylate cyclase activity in synaptosomal membranes of the bovine caudate nucleus.

Compounds	K_a (μM)	Relative maximal activity (%)
Dopamine + GTP	11·9 ± 1·2	190 ± 15*
ADTN + GTP	23·9 ± 3·6	185 ± 21*
Noradrenaline + GTP	40·5 ± 8·6	165 ± 29*
Dopamine without GTP	33·5 ± 4·9	140 ± 10*
ESNN	0·057 ± 0·01	300 ± 28**
ESN	0·355 ± 0·08	220 ± 25**
BrESN	~0·050	160 ± 22**
DHESN	~0·100	140 ± 20**
DHEC	~0·500	135 ± 15**
Lisuride	~5·000	122 ± 12**

K_a—activation constant, i.e. concentration of a stimulant causing half maximal stimulation. It was calculated on the basis of dose-response curves obtained with dopamine agonists (concentration range 10^{-7} to 10^{-3} M) and ergot alkaloid derivatives (concentration range 10^{-9} to 10^{-4} M) using a Lineweaver-Burk equation. Four separate experiments done in triplicate were performed.

* Control value for cAMP formation is 98 ± 12 pmol (mg protein)$^{-1}$ min^{-1}.;

** Basal adenylate cyclase activity is 105 ± 8 pmol (mg protein)$^{-1}$ min^{-1}.

Table 1 shows that dopamine was the most efficient agonist in stimulating the activity of adenylate cyclase in the membrane system examined. The addition of GTP to the incubation mixture enhanced significantly the response, demonstrating the dependence of adenylate cyclase stimulation on guanine nucleotides. ADTN and noradrenaline exerted a prominent stimulant effect, whilst apomorphine and isoprenaline were almost inactive.

Examination of the effect of different dopamine antagonists on adenylate cyclase activity previously stimulated by 50 μM dopamine (180 ± 11 pmol cAMP (mg protein)$^{-1}$ min^{-1}), performed in three separate experiments done in triplicate, gave −log IC50 values of 7·2 ± 0·3, 7·1 ± 0·4 and 6·5 ± 0·6, for (+)-butaclamol, haloperidol and chlorpromazine, respectively. These data show that (+)-butaclamol was the most active inhibitor of dopamine-sensitive adenylate cyclase activity, while (−)-butaclamol and sulpiride did not inhibit. Haloperidol and chlorpromazine also inhibited the response of this enzyme, but to a lesser extent than (+)-butaclamol.

Azuma & Oshino (1980) demonstrated that ergot alkaloids used in higher concentrations inhibited dopamine-dependent adenylate cyclase previously stimulated by dopamine, i.e. they behaved as mixed agonists-antagonists. The effect of ergot alkaloids from ergosine series, as well as alkaloids commonly used, on the activity of dopamine-sensitive adenylate cyclase in

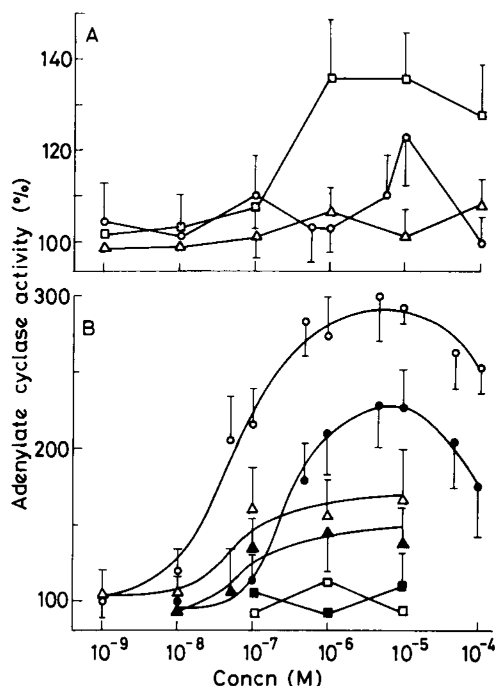

FIG. 1. Stimulation of adenylate cyclase activity bv ergot alkaloids. A: Lisuride (○); DHEC (□): DrEC (△). B: Ergosinine (○); Ergosine (●); BrESN (△); DHESN (▲); BrDHESN (□); DHESNN (■). Basal adenylate cyclase activity is 105 ± 8 pmol (mg protein)$^{-1}$ min^{-1}. Each point represents the mean ± s.e.m. of four separate experiments done in triplicate. Statistical analyses of the data using student's t-test gave the following values for significance: $P < 0{\cdot}05$, $P < 0{\cdot}001$, $P < 0{\cdot}001$, $P < 0{\cdot}02$, $P < 0{\cdot}05$ and $P > 0{\cdot}1$, for DHEC, ergosinine, ergosine, BrESN, DHESN and lisuride, respectively.

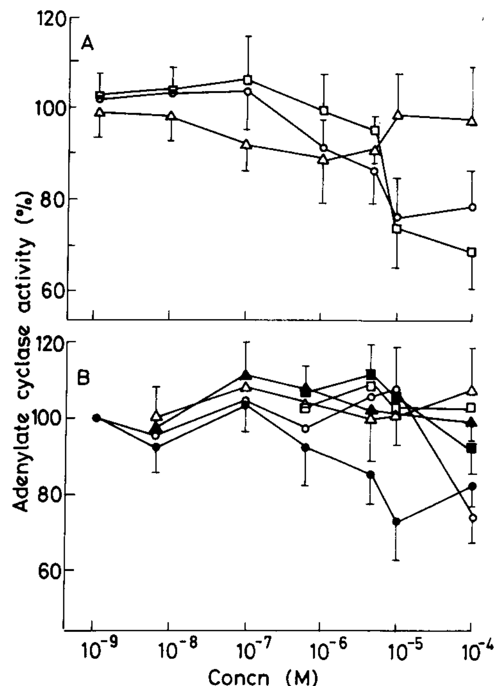

FIG. 2. Inhibition of dopamine-stimulated adenylate cyclase activity by increasing concentrations of ergot alkaloid derivatives. A: Lisuride (○); DHEC (□); BrEC (△). B: Ergosinine (○); ergosine (●); BrESN (△); DHESN (▲); BrDHESN (□); DHESN (■). Adenylate cyclase activity stimulated by 50 μM dopamine is 172 ± 9 pmol cAMP (mg protein)$^{-1}$ min^{-1}. The results are the mean ± s.e.m. of four separate experiments performed in triplicate with standard errors indicated by vertical bars. Statistical analyses of the data using Student's t-test gave the following values for significance: $P < 0{\cdot}02$, $P < 0{\cdot}01$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$, for lisuride, DHEC, ergosine and ergosinine, respectively.

the bovine caudate nucleus previously activated by 50 μM dopamine, is depicted in Fig. 2.

Ergosine, ergosinine, DHEC and lisuride inhibited dopamine-stimulated adenylate cyclase (Fig. 2), while the other ergot alkaloid derivatives did not.

The effects of optimal stimulatory concentrations of ergosine and ergosinine (5×10^{-6} mol litre^{-1}) in the absence and in the presence of 50 μM GTP on adenylate cyclase were examined. Basal adenylate cyclase activity calculated on the basis of these data obtained in four separate experiments done in triplicate, was 110 ± 15 pmol cAMP (mg protein)$^{-1}$ min^{-1}. The stimulation achieved by ergosine and ergosinine in the absence of GTP was 198 ± 18% and 228 ± 46%, respectively. Addition of GTP enhanced the response of adenylate cyclase to 290 ± 50% for ergosine and to 375 ± 25% for ergosinine. These results show that GTP represents a necessary factor for stimulation of adenylate cyclase by the ergosines used, i.e. it was GTP-dependent.

Discussion

Studies on the effects of different catecholamines, dopamine agonists and antagonists on adenylate cyclase

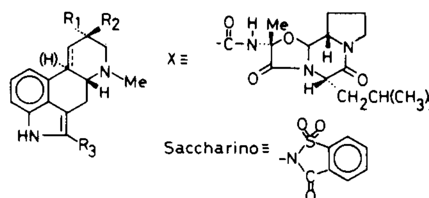

FIG. 3. Schematic representation of the chemical structure of ergot alkaloids from ergosine series examined. Ergosine: $^{9(10)}\Delta$; $R_1 = R_3 = H$; $R_2 = X$. BrESN: $^{9(10)}\Delta$; $R_1 = H$; $R_2 = X$; $R_3 = Br$. SacchESN: $^{9(10)}\Delta$; $R_1 = H$; $R_2 = X$; $R_3 =$ saccharino. DHESN: $R_1 = R_3 = H$; $R_2 = X$. BrDHESN: $R_1 = H$; $R_2 = X$; $R_3 = Br$. Ergosinine: $^{9(10)}\Delta$; $R_1 = X$; $R_2 = R_3 = H$. SacchESNN: $^{9(10)}\Delta$; $R_1 = X$; $R_2 = H$; $R_3 =$ saccharino. DHESNN: $R_1 = X$; $R_1 = R_3 = H$.

(EC 4.6.1.1) activity in canine caudate nucleus, clearly demonstrated the dopamine-dependence of this enzyme (Sano et al 1979a). It was also shown that stimulation of this enzyme was GTP-dependent (Sano et al 1979b; Hoffman 1979). Our results presented here agree well with the data of these authors and demonstrate that we

have been dealing with dopamine-sensitive, GTP-dependent adenylate cyclase in the membranes of the bovine caudate nucleus.

Among the ergot alkaloids from the ergosine series, ergosine and ergosinine had a strong stimulant effect on the activity of adenylate cyclase. Examination of the structure/dopaminergic activity relationship showed that modifications of these molecules (Fig. 3) such as: isomerization in position 8, hydrogenation of $\Delta^{9(10)}$-double bond, or introduction of bromine into position 2 of the molecule, lead to a significant decrease of stimulation of adenylate cyclase.

Introduction of a saccharino group into position 2 of the molecule, caused total loss of stimulatory activity (SacchESN and SacchESNN) very probably because of the bulky saccharino residue. However, metabolic transformations of the saccharino group, of the two saccharino compounds examined throughout our studies, might lead to their different behaviour in relation to their dopaminergic activity in an in-vivo system.

Ergosine, ergosinine, DHEC and lisuride were partial dopamine antagonists and when used in higher concentrations inhibited adenylate cyclase activity, similar to earlier findings obtained with dopamine agonists (Azuma & Oshino 1980; Seiler & Markstein 1982).

GTP was a necessary factor for stimulation by both ergosine and ergosinine, indicating the participation of a GTP regulatory subunit of adenylate cyclase in the activation by these compounds. These data suggest a similar, or probably even identical mechanism of adenylate cyclase activation by ergosines to that caused by catecholamines (Lefkowitz et al 1982; Tolkovsky & Levitzki 1981).

Our results show that some ergot alkaloids from the ergosine series act as strong stimulants of dopamine-dependent adenylate cyclase in the membranes of the bovine caudate nucleus. Ergosinine was the most active in spite of the generally accepted view that derivatives of *iso*-lysergic acid do not possess significant biological activity (Brazeau 1975; Berde & Stürmer 1978). The strong stimulant activity of some ergot derivatives from the ergosine series on dopamine-dependent adenylate cyclase activity also suggests a possible wider application of these substances as dopaminergic agonists in the therapy of diseases associated with the dysfunction of the dopaminergic system.

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