Neuropharmacology Vol. 19, pp. 319 to 323
Pergamon Press Ltd 1980. Printed in Great Britain

PRELIMINARY NOTE

EFFECTS OF CHRONIC AND ACUTE TREATMENT OF ANTIPSYCHOTIC DRUGS ON CALMODULIN RELEASE FROM RAT STRIATAL MEMBRANES

M.E. Gnegy and Y.S. Lau

Department of Pharmacology
University of Michigan Medical School
Ann Arbor, Michigan 48109

(*Accepted 17 December 1979*)

SUMMARY

Chronic treatment of rats with the antipsychotic drugs haloperidol and (+)-butaclamol results in supersensitivity of striatal dopamine (DA) receptors. Striatal membranes of these animals have an increased content of an endogenous Ca^{++}-binding protein, calmodulin. Both endogenous and protein kinase-induced release of calmodulin from striatal membranes of the antipsychotic drug-treated animals were found substantially lower than that from saline or (-)-butaclamol-treated rats. Acute treatment with the antipsychotic drugs produced no alterations in calmodulin content or calmodulin release from the membranes. The impaired calmodulin release seen in the chronic antipsychotic drug-treated rats could be associated with the supersensitivity of DA receptors.

INTRODUCTION

THE CYCLIC nucleotide concentration in the brain can be modulated by the endogenous Ca^{++}-binding protein, calmodulin. In the brain, this protein can stimulate both soluble cyclic nucleotide phosphodiesterase activity (Cheung, 1971) and membrane-bound adenylate cyclase activity (Brostrom, Huang, Breckenridge and Wolff, 1975). In this tissue, calmodulin is mostly located in the membrane portion of the cell and can be released from this fraction into the cytosol by phosphorylation of a membrane binding site (Gnegy, Costa and Uzunov, 1976a). Release of calmodulin from striatal membranes reduces the activation of adenylate cyclase by dopamine (DA) (Gnegy, Uzunov and Costa, 1976b). The calmodulin content was increased in the striatal membranes of rats treated chronically with cataleptogenic antipsychotic drugs that produce behavioral supersensitivity to apomorphine (Gnegy, Lucchelli and Costa, 1977a; Gnegy, Uzunov and Costa, 1977b). Moreover, the striatal membranes from these animals had increased basal and DA-sensitive adenylate cyclase activity. A reduction in calmodulin release could lead to its accumulation in the membrane, which in turn could contribute to the greater adenylate cyclase activity and dopaminergic supersensitivity. Therefore, the purpose of this study was to examine whether calmodulin release from the striatal membranes is altered in rats treated chronically with antipsychotic drugs. Calmodulin release was also measured after acute treatment with the drugs which does not result in behavioral supersensitivity to DA.

METHODS

Male, Sprague Dawley rats (150-200g) were injected daily for 20 days with either haloperidol, (+)-butaclamol, (-)-butaclamol (all at 2.5 μmol/kg s.c.) or saline. One week after the end of treatment some of the animals (N=8) were tested for behavioral supersensitivity to a single dose of apomorphine HCl (1.9 μmol/kg s.c.) as described previously

(Gnegy et al., 1977a,b). In accordance with previous results, animals chronically treated with haloperidol and (+)-butaclamol showed greater stereotyped behavior to apomorphine than those treated with saline or (-)-butaclamol.

Animals not used in behavioral studies were sacrificed by decapitation. The striata were removed, homogenized in 9 vol of 0.32M sucrose and then spun at 20,000 x g for 30 min. The membrane pellets were resuspended in 50 mM Pipes (piperazine-N,N'-bis [2-ethanesulfonic acid]) buffer, pH 7.0. Calmodulin release was measured in an assay containing, in a final volume of 100 µl: 50 mM Pipes buffer, pH 7.0, 10 mM magnesium chloride, 5 µg of partially purified membrane-derived protein kinase from bovine brain (Uno, Ueda and Greengard, 1977), 80-100 µg of striatal membrane protein and 25 µM ATP. The reaction was carried out for 5 min at 30°C and was stopped by plunging the tubes into ice and centrifuging them immediately at 20,000 x g for 30 min. An aliquot of the supernatant was heated at 95°C for 1 min and the calmodulin content was determined using the phosphodiesterase assay as described previously (Gnegy et al., 1976a). The calmodulin content in the particulate fraction (containing 0.5-2 µg protein) of the striatum was similarly determined, except the membrane was initially solublized in 1% Lubrol PX. The calmodulin content was measured in nanograms using a standard curve obtained with highly purified calmodulin prepared from bovine brain (Klee, 1977). Protein was measured by the method of Lowry, Rosenbrough, Farr and Randall (1951), using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The release of calmodulin from the striatal membranes of rats treated chronically with haloperidol, (+)-butaclamol, (-)-butaclamol or saline was measured. As Figure 1 shows there was a substantial endogenous release of calmodulin from the striatal membranes of saline-treated rats that occurred in the presence of Mg^{++} and ATP. The addition of 5 µg of membrane-derived protein kinase elicited a further release of calmodulin. The calmodulin release using striatal membranes from the haloperidol-treated rats was very different from that of control animals. In this case the endogenous calmodulin release was sharply decreased to only 30% of the control value. Calmodulin can be further released by protein kinase in the membranes of haloperidol-treated rats but not to control levels.

The results obtained using rats which were chronically treated with (+)-butaclamol are very similar to those of the haloperidol-treated rats (Figure 2). The endogenous release from striatal membranes of (-)-butaclamol-treated rats was markedly less (44% decrease) than that from saline controls. The amount of calmodulin released upon addition of exogenous protein kinase was also lower than the control value (Figure 2). The endogenous release and protein kinase stimulated release from the membranes of (+)-butaclamol-treated animals were also significantly different from that of the (-)-butaclamol-treated animals. On the other hand, in all respects the values for (-)-butaclamol were similar to control values.

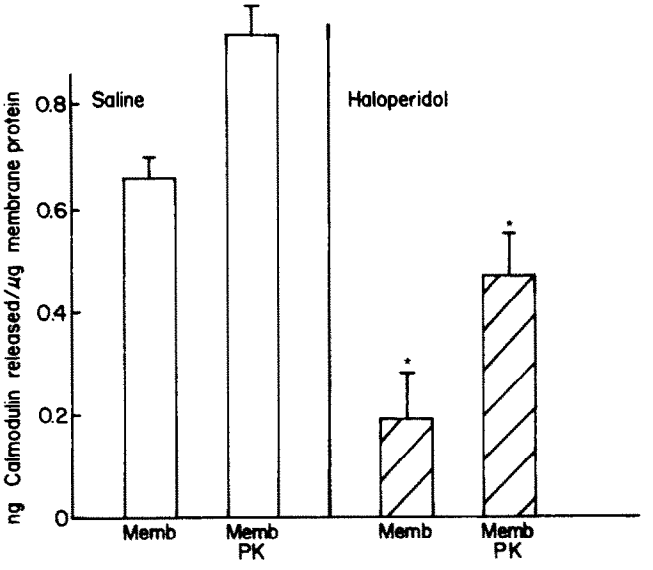

Figure 1. Calmodulin release from striatal membranes of rats treated chronically with saline and haloperidol. Animals were injected daily for 20 days with haloperidol (2.5 µmol/kg s.c.) or saline. One week after stopping chronic injections the rats were sacrificed and calmodulin release was measured as described in Methods. PK=5 µg of protein kinase in the assay. Bars represent mean values for 8 rats ± S.E.M. *$p < 0.001$ when compared with saline-treated rats.

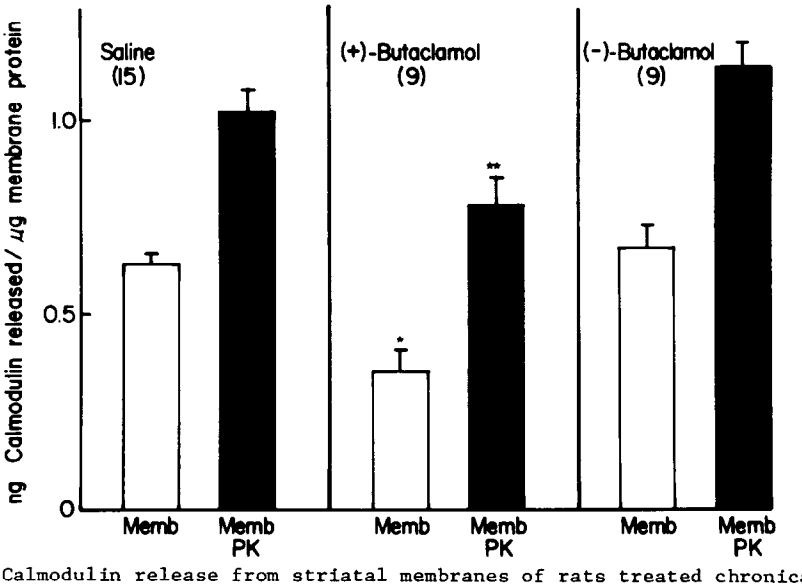

Figure 2. Calmodulin release from striatal membranes of rats treated chronically with saline, (+)-butaclamol and (-)-butaclamol. Animals were injected daily for 20 days with (+)-butaclamol, (-)-butaclamol (2.5 µmol/kg s.c.) or saline. One week after stopping chronic injections the rats were sacrificed and calmodulin release was measured as described in Methods. PK=5µg of protein kinase in the assay. Bars represent mean values ± S.E.M. The number of animals is given in parenthesis. *$p < 0.01$ with respect to saline-treated controls and (-)-butaclamol-treated rats. **$p < 0.05$ with respect to saline-treated controls and (-)-butaclamol-treated rats.

The reduction in calmodulin release from striatal membranes of supersensitive rats is not simply due to a lack of calmodulin in the membranes. The haloperidol-treated animals had a 28% increase in their striatal membrane content of calmodulin (5.1 ± 0.1 ng/µg protein in haloperidol-treated animals, 4.0 ± 0.2 ng/µg protein in saline-treated rats, $p < 0.01$, N=8). Therefore, the percentage of calmodulin that could be released from the membranes of haloperidol-treated rats (9%) was nearly three-fold less than that from membranes of control rats (24%), (N=8).

Calmodulin release after acute treatment with a single dose of haloperidol, (+)-butaclamol, (-)-butaclamol (all at 2.5 µmol/kg s.c.) or saline was also studied. The animals were sacrificed one hour after the drug treatment. At the time of sacrifice, the haloperidol-treated and (+)-butaclamol-treated rats exhibited stuporous behavior which was not seen in the (-)-butaclamol or saline-treated animals.

The release of calmodulin from the striatal membrane of animals acutely treated with haloperidol and (+)-butaclamol was not different from that of saline or (-)-butaclamol-treated rats (Table 1). Furthermore, there was no significant change in membrane calmodulin content of the acutely drug-treated rats as compared to the saline controls. (Table 1).

As demonstrated in this study, rats treated chronically with haloperidol and (+)-butaclamol showed a decreased calmodulin release from that of saline of (-)-butaclamol controls. Furthermore, acute treatment of rats with the drugs haloperidol, (+)-, or (-)-butaclamol produced no change in striatal calmodulin content or its release from membranes. An impaired ability to release calmodulin in these chronic antipsychotic drug-treated animals could lead to its eventual accumulation in the membrane. This latter phenomenon could cause an increase in DA-sensitive adenylate cyclase activity (Gnegy et al., 1977a,b), which would contribute to the dopaminergic supersensitivity in the striatum produced by chronic DA receptor blockade.

The greatest difference between the drug-treated and control groups was in the endogenous calmodulin release. The mechanism of this endogenous release is unknown at the present time. Preliminary observations, however, suggest that this endogenous release is regulated by a Ca^{++}-dependent process, possibly Ca^{++}-dependent protein kinase activity. Since the primary function of calmodulin is to bind calcium, it is logical that the calcium concentration itself could be a modulator of the binding and "releasability" of calmodulin. The significant decrease in endogenous release of calmodulin in the supersensitive animals could reflect a change in calcium concentration or sequestration in the membranes of these

TABLE 1

Calmodulin content and its release from striatal membranes of rats acutely treated with saline, haloperidol, (+)-butaclamol, and (-)-butaclamol

Treatment	N	Membrane Calmodulin Content	Calmodulin release from membrane -protein kinase	+protein kinase
			ng calmodulin/μg membrane protein ± S.E.M.	
Expt. I				
Saline	6	3.7 ± 0.3	1.2 ± 0.1	1.6 ± 0.1*
Haloperidol	8	4.0 ± 0.2	1.2 ± 0.1	1.8 ± 0.1*
Expt. II				
Saline	4	3.8 ± 0.4	1.2 ± 0.1	1.9 ± 0.1*
(+)-Butaclamol	8	3.4 ± 0.3	1.1 ± 0.1	2.0 ± 0.1*
(-)-Butaclamol	8	3.9 ± 0.2	1.2 ± 0.1	1.9 ± 0.1*

Animals were given one injection of haloperidol, (+)-butaclamol, (-)-butaclamol (2.5 μmol/s.c.) or saline. The animals were sacrificed one hour after treatment and the calmodulin release was measured as described in METHODS. *The increase in release by protein kinase is significant at $p < 0.05$. All values among the treatments are not statistically different.

animals. Exogenously added protein kinase did not release the calmodulin from the membranes of the chronic antipsychotic drug-treated rats to the same levels as those seen in the saline-treated controls. Since we are adding an adequate amount of protein kinase in the assay, this indicates that perhaps some of the substrate for the reaction in the former groups is present in a less reactive state.

The results from this study are important because they can begin to explain intermembrane molecular events that occur during supersensitivity to the neurotransmitter, DA. Supersensitivity and subsensitivity of catecholamine-linked adenylate cyclase could be due to many factors and be exhibited at many levels such as the receptor, catalytic activity of adenylate cyclase, any intermembrane "linking" factors (such as GTP-binding proteins or calmodulin) between receptor and cyclase, or phosphodiesterase and protein kinase activity. All of these factors have been shown to be involved in various examples of supersensitivity and subsensitivity to catecholamines (see review by Gnegy and Costa, 1980).

ACKNOWLEDGMENTS

This work was funded by NSF grant No. BNS-78-06964 and a Phoenix Memorial Project Grant from the University of Michigan, Ann Arbor, Michigan. It has been partially presented in abstract form at the Spring meeting of the Federation of American Societies for Experimental Biology, Dallas, Texas, 1979.

REFERENCES

Brostrom, C.O., Huang, Y.C., McL. Breckenridge, B. and Wolff, D.J. (1975). Identification of a calcium-binding protein as a calcium-dependent regulator of brain adenylate cyclase. Proc. Natl. Acad. U.S.A. 72:64-68.

Cheung, W.Y. (1971). Cyclic 3',5'-nucleotide phosphodiesterase. Evidence for an properties of a protein activator. J. Biol. Chem. 254:2859-2869.

Gnegy, M.E. and Costa, E. (1979). Catecholamine receptor supersensitivity and subsensitivity in the central nervous system. In: Essays in Neurochem. and Neuropharm. (Youdim, M.B.H., Lovenberg, W., Sharman, D.F. and Lagnado, J.R., Eds.), Vol. 4, Wiley and Sons, New York, pp. 249-282.

Gnegy, M.E., Costa, E. and Uzunov, P. (1976a). Regulation of transsynaptically elicited increase of 3':5'-cyclic AMP by endogenous phosphodiesterase activator. Proc. Natl. Acad. Sci. U.S.A. 73:352-355.

Gnegy, M.E., Lucchelli, A. and Costa, E. (1977a). Correlation between drug-induced supersensitivity of dopamine striatal dependent mechanisms and the increase in striatal content of the Ca^{++}-regulated protein activator of cAMP phosphodiesterase. Naunyn-Schmiedebergs. Arch. Pharmacol. 301:121-127.

Gnegy, M.E., Uzunov, P. and Costa, E. (1976b). Regulation of dopamine stimulation of striatal adenylate cyclase by an endogenous Ca^{++}-binding protein. Proc. Natl. Sci. U.S.A. 73:3887-3890.

Gnegy, M. Uzunov, P. and Costa, E. (1977b). Participation of an endogenous Ca^{++}-binding protein activator in the development of drug-induced supersensitivity of striatal dopamine receptors. J. Pharm. and Exptl. Therap. 202:558-564.

Klee, C.B. (1977). Conformation transition accompanying the binding of Ca^{++} to the protein activator of 3',5'-cyclic adenosine monophosphate phosphodiesterase. Biochemistry 16:1017-1024.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Uno, I., Ueda, T. and Greengard, P. (1977). Adenosine 3':5'-monophosphate-regulated phosphoprotein system of neuronal membranes. II. Solubilization, purification and some properties of an endogenous adenosine 3':5'-monophosphate-dependent protein kinase. J. Biol. Chem. 252:5164-5174.