the present investigation that this MADH is another enzyme which takes part in the physiological inactivation of biogenic amines. Of course, further work in this direction is essential, especially to elucidate its actual role in connection with monoamine metabolism.

Acknowledgements—This work was financially supported by the Atomic Energy Commission, India (S. K. Ghosh was a JRF to AEC). The authors are grateful to Dr. B. Mukherji and Dr. N. Chakraborty for their constant inspiration throughout the work.

Indian Institute of Experimental Medicine S. K. GHOSH*
India S. R. GUHA

REFERENCES

1. E. A. Zeller and S. Sarkar, *J. biol. Chem.* **237**, 2333 (1962).
2. G. M. Barbato and L. G. Abood, *Biochem biophys. Acta* **67**, 531 (1964).
3. S. R. Guha, *Biochem. Pharmac.* **15**, 161 (1966). (1959).
5. B. B. Brodie, S. Spector and P. A. Shore, *Ann. N.Y. Acad. Sci.* **80**, 609 (1959).
6. S. Spector, *Ann. N.Y. Acad. Sci.* **107**, 856 (1963).
7. H. Green and J. L. Sawyer, *Proc. Soc. exp. Biol. Med.* **104**, 153 (1960).
8. S. M. Hess and S. Udenfriend, *J. Pharmac. exp. Ther.* **127**, 178 (1959).
9. H. Blaschko, *Pharmac. Rev.* **4**, 415 (1952).
10. A. N. Davison, *Physiol. Rev.* **38**, 721 (1958).
11. J. R. Lagnado and T. L. Sourkes, *Can. J. Biochem. Physiol.* **34**, 1095 (1956).
12. J. R. Lagnado and T. L. Sourkes, *Experientia*, **13**, 476 (1957).
13. M. I. J. Dianzani, *Nature, Lond.* **171**, 125 (1953).
14. N. Robinson, *J. neurochem.* **14**, 1083 (1967).
15. S. R. Guha and S. K. Ghosh, *Biochem. Pharmac.* **19**, 2929 (1970).
16. S. R. Guha, A. R. Bose and S. K. Ghosh, *Indian J. Biochem. Biophys.* **8**, 170 (1971).
17. S. K. Ghosh and S. R. Guha, *J. Neurochem.* **19**, 229 (1972).
18. S. R. Guha, P. S. Basu and K. K. Sen, *Acta. biol. med. germ.* **18**, 417 (1972).
19. A. L. Green and T. M. Haughton, *Biochem. J.* **78**, 173 (1961).
20. W. W. Umbreit, R. H. Burris and J. F. Stauffer (eds), in *Mamometric Techniques*, p. 130. Burgess Publishing Co., U.S.A. (1957).
21. S. K. Ghosh and S. R. Guha (in preparation).
22. H. C. Kimand and A. D'Iorio, *Can. J. Biochem.* **46**, 295 (1968).
23. G. G. S. Collins, M. B. H. Youdim and M. Sandler, *FEBS. Lett.* **1**, 215 (1968).
24. M. H. Brooke and W. K. Engel, *Neurology* **16**, 986 (1966).
25. H. Weissbach, B. G. Redfield and G. G. Glenner, *J. Histochem. Cytochem.* **5**, 601 (1957).
26. L. Sierens and A. D'Iorio, *Can. J. Biochem.* **48**, 659 (1970).
27. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
28. R. F. Squires, *Biochem. Pharmac.* **17**, 1401 (1968).

* Present Address: National Institute of Occupational Health, Ahmedabad-380016, Gujaiat, India.

Biochemical Pharmacology, Vol. 27, pp. 114–116. Pergamon Press, 1978. Printed in Great Britain.

Effects of chlorpromazine and DL-amphetamine on calcium uptake by adrenal chromaffin cell membrane

(*Received* 24 *January* 1977; *accepted* 8 *March* 1977)

Biochemical investigations have shown that plasma membrane of adrenal medulla is concentrated in the microsomal fraction [1, 2]. Electron microscopy has shown that vesicles form from this plasma membrane-rich fraction [1, 2], and biochemical evidence suggests that these plasma membrane vesicles take up calcium in the presence of ATP [2]. This calcium transfer mechanism may be of importance in regulating calcium-mediated secretion. Agents that modify this transfer mechanism may also modify secretion in the intact organ. Recent evidence suggests that caffeine and certain inorganic cations may act, at least in part, by interfering with this plasma membrane calcium transfer mechanism [3]. Caffeine, Zn^{2+}, Cd^{2+} and Hg^{2+} produce a relatively prolonged catecholamine release from perfused adrenal medulla [4, 5]. These agents probably cause secretion by mobilizing intracellular calcium [4] and by inhibiting calcium extrusion from the adrenal medullary cell [3].

It has also been proposed that chlorpromazine and amphetamine evoke catecholamine release from the adrenal medullary cell by mobilizing intracellular calcium pools [4]. The purpose of the present investigation was to determine if chlorpromazine and amphetamine also inhibit adrenal medullary plasma membrane calcium transport.

Bovine adrenal glands were obtained from a local slaughterhouse and carried on ice to the laboratory and used within 2 hr post-mortem. Approximately 5 g of medullary tissue was dissected free from the cortex and homogenized in 10 vol. of 0.32 M sucrose using a conical all-glass homogenizer (Duall tissue grinder, Kontes Glass Co.). The homogenate was centrifuged at 800 g_{max} for 10 min to separate nuclei and cell debris. The supernatant obtained was centrifuged at 27,000 g_{max} for 10 min to separate mitochondria and chromaffin granules. This supernatant was centrifuged at 105,000 g_{max} for 60 min. The final pellet which represented purified microsomes was resuspended in 5 ml of 0.32 M sucrose.

For Ca^{2+}-stimulated adenosinetriphosphatase (Ca^{2+}-ATPase) activity, a 0.1-ml aliquot of the sample was made 0.1 per cent with respect to sodium desoxycholate and incubated for 20 min at 37° in a Dubnoff metabolic shaker

with $CaCl_2 \cdot 2H_2O$, 2.5 mM; ATP-disodium salt, 4 mM; and 100 μmoles Tris–HCl, pH 7.4, in a 3-ml volume. Calcium-stimulated inorganic phosphate liberation was measured by the method of Lowry and Lopez [6].

$^{45}Ca^{2+}$ uptake was studied at 37° for 10 min in a 3-ml incubation mixture containing Tris–HCl (pH 7.4), 30 μmoles; ATP-dimagnesium salt, 9 μmoles; ammonium oxalate, 15 μmoles; $^{45}Ca^{2+}$, 0.4 μCi; calcium, 0.2 mM; and 0.1 ml of the microsomal fraction. All experiments were performed in the presence and absence of ATP. The incremental increase in the presence of ATP was considered ATP-mediated $^{45}Ca^{2+}$ uptake.

Millipore filters (0.45 μm) were soaked in 250 mM KCl solution and then washed with 10 ml of distilled water prior to sample filtration to reduce $^{45}Ca^{2+}$ binding. After incubation the samples were filtered through the 0.45-μm Millipore filters under suction. The particulate matter on the filters was then washed with 10 ml 0.32 M sucrose. The filters were then dried and placed in glass counting vials. Fifteen ml of scintillation fluid [0.6% 2,5-diphenyloxazole (PPO) and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in 1:1 toluene and 2 ethoxyethanol] was added and the $^{45}Ca^{2+}$ counted in a Packard Tricarb scintillation counter. Counting efficiency was determined using an external standard.

Total protein was estimated colorimetrically by the biuret method [7].

The data in Table 1 are reported as ATP-mediated uptake of $^{45}Ca^{2+}$. Nonspecific binding of $^{45}Ca^{2+}$ in the absence of ATP was subtracted from the total concentration of $^{45}Ca^{2+}$ in the presence of ATP. The resulting increment in $^{45}Ca^{2+}$ content is referred to as ATP-mediated uptake. Ammonium oxalate was used as the permiant anion to facilitate $^{45}Ca^{2+}$ transport. Oxalate has been previously shown to augment the uptake of $^{45}Ca^{2+}$ into vesicles of adrenal medullary microsomes [2].

Chlorpromazine, at a concentration range of 0.01 to 1.0 mM, did not significantly alter ATP-mediated $^{45}Ca^{2+}$ uptake. However, amphetamine in the same concentration ranges tended to increase $^{45}Ca^{2+}$ uptake. This augmented uptake was inversely proportional to the concentration of amphetamine used. Amphetamine, 0.01 mM and 0.1 mM, produced a 71 and 25 per cent increase in $^{45}Ca^{2+}$ uptake, respectively, while 1.0 mM amphetamine did not significantly alter the uptake process as compared to controls.

Neither chlorpromazine nor amphetamine in concentration ranges of 0.01 to 1.0 mM altered Ca^{2+}–ATPase activity (Table 2). Although chlorpromazine at a concentration of 1.0 mM produced an apparent inhibition of Ca^{2+}–ATPase activity, this depression was not significantly different from control values.

Nonspecific binding of $^{45}Ca^{2+}$ in the absence of ATP was significantly altered by both chlorpromazine and amphetamine (Table 3). Chlorpromazine, 1.0 mM, produced a 31 per cent depression of $^{45}Ca^{2+}$ binding, but binding was not altered by chlorpromazine at lower concentrations (0.01 and 0.1 mM). Amphetamine, on the other hand, potentiated $^{45}Ca^{2+}$ nonspecific membrane binding. Amphetamine, 0.01 and 1.0 mM, produced approximately a 43 per cent increase in $^{45}Ca^{2+}$ binding. Amphetamine at a concentration of 0.1 mM produced an apparent 34 per cent increase in $^{45}Ca^{2+}$ binding but this increase was not significant as compared to controls.

Physiologic release of catecholamines from the adrenal medulla is known to require extracellular calcium [8]. Agents such as potassium and acetylcholine depolarize the adrenal medullary cell membrane and allow calcium to enter the chromaffin cell to initiate stimulus–secretion coupling [8]. Removal of extra-cellular calcium eliminates the ability of these agents to cause secretion. Caffeine,

Table 1. Effects of chlorpromazine and DL-amphetamine on ATP-mediated $^{45}Ca^{2+}$ uptake by adrenal medullary cell membrane*

	ATP-mediated $^{45}Ca^{2+}$ uptake (μmoles/mg protein)†	Per cent of control
Control (N = 5)	11.14 ± 2.70	
Chlorpromazine		
1.0 mM (N = 5)	9.54 ± 2.19	86 ± 19.7
0.1 mM (N = 5)	12.54 ± 3.52	113 ± 31.6
0.01 mM (N = 5)	12.26 ± 1.50	110 ± 13.5
Control (N = 4)	11.49 ± 1.09	
Amphetamine		
1.0 mM (N = 4)	11.95 ± 0.42	104 ± 3.7
0.1 mM (N = 4)	14.37 ± 0.38‡	125 ± 3.3
0.01 mM (N = 4)	19.63 ± 3.94‡	171 ± 34.3

* Values represent mean ± S. E.

† Statistical significance was determined using the Student *t*-test.

‡ Significantly different from controls ($P < 0.05$).

Table 2. Effects of chlorpromazine and DL-amphetamine on Ca^{2+}-ATPase activity of adrenal medullary cell membrane*

	Ca^{2+}–ATPase activity (μmoles Pi/20 min/mg protein)†	Per cent of control
Control (N = 5)	4.50 ± 0.87	
Chlorpromazine		
1.0 mM (N = 5)	3.22 ± 0.33	72 ± 7.4
0.1 mM (N = 5)	5.20 ± 0.68	116 ± 15.2
0.01 mM (N = 5)	4.57 ± 0.58	102 ± 12.3
Control (N = 4)	4.05 ± 0.44	
Amphetamine		
1.0 mM (N = 4)	3.98 ± 0.46	99 ± 11.4
0.1 mM (N = 4)	3.89 ± 0.39	96 ± 9.6
0.01 mM (N = 4)	3.73 ± 0.34	92 ± 8.4

* Values represent mean ± S. E.

† Statistical significance was determined using the Student *t*-test.

Table 3. Effects of chlorpromazine and DL-amphetamine on $^{45}Ca^{2+}$ nonspecific binding by adrenal medullary cell membrane*

	$^{45}Ca^{2+}$ nonspecific binding (μmoles/mg protein)†	Per cent of control
Control (N = 5)	8.56 ± 1.46	
Chlorpromazine		
1.0 mM (N = 5)	5.93 ± 0.50‡	69 ± 5.8
0.1 mM (N = 5)	7.34 ± 1.16	86 ± 15.8
0.01 mM (N = 5)	8.18 ± 1.57	96 ± 18.2
Control (N = 4)	4.97 ± 0.44	
Amphetamine		
1.0 mM (N = 4)	7.26 ± 1.75‡	143 ± 35.2
0.1 mM (N = 4)	6.66 ± 1.22	134 ± 24.6
0.01 mM (N = 4)	7.10 ± 0.87‡	143 ± 17.5

* Values represent mean ± S. E.

† Statistical significance was determined using the Student *t*-test.

‡ Significantly different from control ($P < 0.05$).

chlorpromazine and amphetamine have also been shown to cause catecholamine secretion from the adrenal medulla [4], but, unlike potassium and acetylcholine, these drugs will stimulate release in the absence of extracellular calcium. It has been suggested that these pharmacological agents can evoke catecholamine secretion by mobilizing different calcium pools within the adrenal medullary cell [4].

Caffeine also decreases ATP-mediated $^{45}Ca^{2+}$ uptake by plasma membrane-rich subfractions of adrenal medullary microsomes. Since it has been reported that this calcium pump is localized in the plasma membrane [1, 2], it was suggested that caffeine may possess the capacity to interfere with calcium removal from the cell and thus inhibit termination of the secretory response [3]. Studies with chlorpromazine and amphetamine in this investigation suggest that these agents do not depress the chromaffin cell membrane calcium pump. Amphetamine increased ATP-mediated $^{45}Ca^{2+}$ uptake by plasma membrane-rich fractions of adrenal medullary microsomes. This increase was not associated with any change in Ca^{2+}-ATPase activity. This may suggest that amphetamine may exert a pharmacological action directly on the ultrastructure of the plasma membrane in a manner which does not involve the ATP-dependent Ca^{2+} pump reported by Leslie and Borowitz [2].

The potentiation of Ca^{2+} uptake by amphetamine appears to be inversely proportional to the concentration. This type of response to amphetamine has been previously reported in the literature [9]. Amphetamine at a dosage of 2.5 mg/kg significantly increases endogenous mouse brain dopamine levels, while amphetamine (15 mg/kg) significantly reduces brain dopamine concentrations.

Chlorpromazine has been reported to decrease $^{45}Ca^{2+}$ uptake in the mitochondrial fraction of rat brain [10]. In the sarcoplasmic reticulum of rabbit skeletal muscle, chlorpromazine activates, while chlorpromazine free radical inhibits, the calcium transport system [11]. In this investigation chlorpromazine had no significant effect on $^{45}Ca^{2+}$ uptake by plasma membrane. In addition, no significant change was seen in Ca^{2+}-ATPase activity.

Nonspecific $^{45}Ca^{2+}$ binding in the absence of ATP was significantly decreased with 1.0 mM chlorpromazine. This may be due to the potent surface activity of chlorpromazine [12]. More recent work with erythrocyte ghosts [13] and with simulated membranes [14] suggest that chlorpromazine may competitively displace Ca^{2+} from binding sites and alter membrane structure, making fewer binding sites available.

In conclusion, it appears that chlorpromazine has no effect on the ATP-dependent Ca^{2+} uptake process of adrenal medullary plasma membrane, but does, in high concentrations, depress nonspecific $^{45}Ca^{2+}$ binding to membranes of the microsomal fraction. Amphetamine increases ATP-dependent $^{45}Ca^{2+}$ uptake but there is no corresponding change in Ca^{2+}-ATPase activity. This suggests that the amphetamine action is not on the ATP-dependent Ca^{2+} pump.

Acknowledgement—This project was supported by a grant from the University Research Institute, The University of Texas at Austin.

Department of Pharmacology, College of Pharmacy and * *Department of Health, Physical Education and Recreation, The University of Texas at Austin, Austin, TX 78712, U.S.A.*

STEVEN W. LESLIE
STEVE V. ELROD
HUGH W. BONNER*

REFERENCES

1. M. S. Nijjar and J. N. Hawthorne, *Biochim. biophys. Acta* **367**, 190 (1974).
2. S. W. Leslie and J. L. Borowitz, *Biochim. biophys. Acta* **394**, 227 (1975).
3. S. W. Leslie and J. L. Borowitz, *Res. Commun. Chem. Path. Pharmac.* **11**, 413 (1975).
4. R. G. Rahwan, J. L. Borowitz and T. S. Miya, *J. Pharmac. exp. Ther.* **184**, 106 (1973).
5. D. T. Hart and J. L. Borowitz, *Archs int. Pharmacodyn. Thér.* **209**, 94 (1974).
6. O. H. Lowry and J. A. Lopez, *J. biol. Chem.* **162**, 421 (1946).
7. E. Layne, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. III, p. 447. Academic Press, New York (1957).
8. W. W. Douglas, T. Kanno and S. R. Sampson, *J. Physiol., Lond.* **191**, 107 (1967).
9. W. H. Riffee and M. C. Gerald, *Archs int. Pharmacodyn. Thér.* **219**, 70 (1976).
10. S. Tjioe, N. Haugaard and C. P. Bianchi, *J. Neurochem.* **18**, 2171 (1971).
11. M. A. O'Callaghan and P. F. Duggan, *Biochem. Pharmac.* **24**, 563 (1975).
12. P. M. Seeman and H. S. Bialy, *Biochem. Pharmac.* **12**, 1181 (1963).
13. P. M. Seeman and W. O. Kwant, *Biochim. biophys. Acta* **183**, 530 (1969).
14. R. N. Schaubman, J. L. Zatz and A. Felmeister, *Abstr. Acad. Pharm. Sci.* **6:2**, 98 (1976).