very close to the minimum dose that prevents the statistically significant increase in MAO activity caused by adrenalectomy.

Because of its activity as a mineralcorticoid, DOCA causes retention of sodium. Giving the adrenalectomized animals an isotonic sodium chloride solution to drink also allows them to take in and utilize more sodium than if they had been drinking water. Thus, DOCA injections and saline to drink both help the adrenalectomized animals with their need for sodium. The combination of DOCA plus saline also prevents the adrenalectomy-induced increase in heart MAO activity. If DOCA prevents the adrenalectomy-induced increase in MAO activity because of its effects on sodium metabolism then decreasing the amount of sodium in the diet should decrease the capacity of DOCA to prevent the increased MAO activity. The 2nd experiment was designed to test this possibility. In this experiment, approximately half of the adrenalectomized animals were given 0.9% saline as their only drinking fluid and the remainder had water to drink and access to an alternate salt source. Approximately half of the animals in each of these groups received daily injections of DOCA. The results of this experiment are shown in figure 3 (norepinephrine as substrate) and figure 4 (phenethylamine as substrate). In the animals that had saline as their only drinking fluid, the enzyme activity increased to 139% of control (norepinephrine substrate) and 140% of control (phenethylamine substrate). In the animals drinking water, the enzyme activity increased to 171% of control with norepinephrine as the MAO substrate and to 160% of control with phenethylamine as the substrate (figs 3 and 4). Thus, the adrenalectomized animals drinking water appeared to have a greater increase in MAO activity than did the animals that had saline to drink. Additionally, daily administration of DOCA prevented the increase in MAO activity in the animals that had 0.9% saline to drink but did not cause a statistically significant suppression of the MAO activity in the animals drinking water. Thus, the dose of DOCA that was minimally effective at preventing the increase in MAO activity in animals drinking saline was ineffective at preventing the MAO activity increase when the animals were drinking water. Since this dose of DOCA suppressed the MAO increase in animals with a high sodium intake but not in animals with a lower sodium intake it is suggested that the action of DOCA on MAO activity may be related to its actions on sodium metabolism.

Other authors have found that daily glucocorticoid administration coupled with saline as the drinking solution prevents the adrenalectomy-induced increases in MAO activity[6,11,12]. The prevention of the increased enzyme activity has been attributed to an effect of the glucocorticoid. The results of the present study suggest that there may be a relationship between sodium intake and the adrenalectomy-induced change in MAO activity. Also, administration of an adrenal steroid, either a mineralocorticoid or a glucocorticoid appears to be necessary to completely prevent the change in the activity of this enzyme. At this time it is unclear whether mineralocorticoids and glucocorticoids suppress the adrenalectomy-induced increase in MAO activity through the same mechanism.

In summary, it can be said that the findings of this study show that mineralocorticoids and sodium together, but not alone prevented the adrenalectomy-induced increases in MAO activity.

1 Acknowledgment. This work was supported in part by a grant from the American Heart Association - Louisiana, Inc.
2 C.P. Richter, Am. J. Physiol. *115*, 155 (1936).
3 C.P. Richter, Endocrinology *29*, 115 (1941).
4 M.J. Fregly and I.W. Waters, Physiol. Behav. *1*, 65 (1966).
5 T.C. Westfall and H. Osada, J. Pharmac. exp. Ther. *167*, 300 (1969).
6 S.S. Sampath and D.E. Clark, Life Sci. *11*, 1037 (1972).
7 J.W. Dailey and T.C. Westfall, Eur. J. Pharmac. *48*, 383 (1978).
8 S. Parvez and H. Parvez, Hormone Res. *5*, 234 (1974).
9 P.M. Caesar, G.G.S. Collins and M. Sandler, Biochem. Pharmac. *19*, 921 (1970).
10 P.C. Waldmeier, A. Delini-Stula and L. Maitre, Naunyn-Schmiedeberg's Arch. Pharmak. *292*, 9 (1976).
11 H. Parvez and S. Parvez, Acta endocr. *73*, 509 (1973).
12 J. Knopp, R. Kvetnansky and M. Torda, Experientia *33*, 586 (1976).

Amantadine modulates phencyclidine binding site sensitivity in rat brain

R. Quirion and Candace B. Pert[1]

Biological Psychiatry Branch, National Institute of Mental Health, Clinical Center, Room 3N256, 9000 Rockville Pike, Bethesda (Maryland 20205, USA), 19 January 1982

Summary. Amantadine, an antiviral drug with various CNS effects, significantly increases the affinity of the [^{3}H] PCP receptor in rat brain. Rimantadine, an analogue of amantadine devoided of CNS effects, does not have any affect on the [^{3}H] PCP receptor. These results may suggest that some of the CNS actions of amantadine are related to an interaction with the PCP receptor.

Amantadine (1-adamantanamine) is an antiviral drug which has been shown, unexpectedly, to improve the symptomatic condition of parkinsonian patients[2,3]. The mechanism of action related to this antiparkinsonism activity is not completely understood, but appears to be due to the dopamine-releasing properties of amantadine[4-6]. Compared to levodopa, amantadine is relatively free of side effects[7]. Interestingly, amantadine may cause severe mental symptoms in patients with a history of psychiatric disorders[7]. Since we have recently shown the existence of [^{3}H] phencyclidine (PCP) receptors in rat brain[8] and because PCP possesses a peculiar profile of psychotomimetic effects[9], we have decided to investigate the possible interaction of amantadine with the PCP binding site.

Materials and methods. Rat olfactory bulb slices were prepared as described before[8]. Frozen slide-mounted sections were preincubated for 15 min in 5.0 mM Tris-HCl, 50 mM sucrose, 20 mM NaCl, pH 7.4 at 0 °C followed by a 45-min incubation in the same buffer (without NaCl), pH 7.4 at 0 °C with various concentrations of [^{3}H] PCP (48 Ci/mmole; New England Nuclear) in the presence of various concentrations of amantadine or its inactive analogue, rimantadine. At the end of the incubation, the slides were transferred sequentially through 6 rinses (30 sec in

each) of 5.0 mM Tris-HCl buffer plus 50 mM sucrose, pH 7.4 at 0 °C plus 1% bovine serum albumin. Binding of [^{3}H] PCP to the tissue slice was quantitated by counting the tissue-laden slide fragment in 10 ml Aquassure scintillation cocktail (New England Nuclear). Specific binding was calculated as the difference in counts bound in the presence and absence of 0.1 mM PCP.

All data analyses comparing amantadine-treated and control [^{3}H] PCP binding were done using a t-test (student distribution) of mean differences.

Results and discussion. As indicated in the table, amantadine significantly increases the affinity (K_D) of [^{3}H] PCP for its binding site in a dose dependant manner. The number of sites (B_{max}) does not appear to be changed by the presence of amantadine in the incubation buffer. Interestingly enough, rimantadine, an analogue of amantadine with antiviral properties, but completely devoid of any CNS effects[10], is also totally inactive on the [^{3}H] PCP binding site.

It is tempting to speculate that may be some of the CNS effects of amantadine are related to an action of this drug on the PCP receptor complex. It is well known that PCP is able to induce the release of dopamine in various conditions[11-13] and acts as a 'non-amphetamine' stimulant of the dopaminergic system[14]. Since the mechanisms of action of amantadine appears to involve the release of dopamine from the pre-synaptic area[4-6], it is possible that both amantadine and PCP act on the same substrate to induce the release of dopamine in the brain. Also, this interaction of amantadine on the PCP binding site may explain some of the CNS effects of this antiviral drug. In fact, it had been reported that both PCP[15] and amantadine[7] induced hallucinations in humans. An other possibility might be the interaction of both PCP and amantadine with the cholinergic system[16], which may result in an increased release of dopamine induced by stimulation of nicotinic receptors[7]. Finally, our results show that [^{3}H] PCP binding sites in rat brain are different from those present in membranes of torpedo ocellata, since amantadine displaced [^{3}H] PCP from its binding site in this system which is probably related to the ion channel of the nicotinic receptor[16].

Effects of amantadine on [^{3}H] PCP binding parameters

Drug	[^{3}H] PCP K_D (nM)	B_{max} (fmole/slice)
Control	52±4.0	10.6±0.9
Amantadine (10 nM)	41±3.0*	11.0±0.8
Amantadine (100 nM)	36±3.0**	9.9±0.7
Rimantadine (100 nM)	54±5.0	11.4±1.0

Numbers are mean±SEM of 3 determinations each in triplicate. *$p<0.05$; **$p<0.01$.

1 Acknowledgments. We thank Dr W.K. Schmidt (E.I. Dupont, Newark, Delaware) for gifts of amantadine and rimantadine. Dr R. Quirion is a fellow of the Medical Research Council of Canada.
2 R.S. Schwab, A.C. England, Jr, D.C. Poskanzer and R.R. Young, J. Am. med. Ass. *208*, 1168 (1969).
3 R.S. Schwab, D.C. Poskanzer, A.C. England and R.R. Young, J. Am. med. Ass. *222*, 792 (1972).
4 R.P. Grelak, R. Clark, J.M. Stump and V.G. Vernier, Science *169*, 203 (1970).
5 P.F. Von Voigtlander and K.E. Moore, Science *174*, 408 (1971).
6 L.O. Farnebo, K. Fuxe, M. Goldstein, B. Hamberger and U. Ungerstedt, Eur. J. Pharmac. *16*, 27 (1971).
7 L.S. Goodman and A. Gilman, eds, in: The pharmacological basis of therapeutics, 5th edn, p.235. Macmillan Press, New York 1975.
8 R. Quirion, R.P. Hammer, Jr, M. Herkenham and C.B. Pert, Proc. natl Acad. Sci. USA *78*, 5881 (1981).
9 G.D. Pearlson, Johns Hopkins med. J. *148*, 25 (1981).
10 V.G. Vernier, personal communication.
11 T.E. Ary and H.L. Komisky, Eur. J. Pharmac. *61*, 401 (1980).
12 S.P. Bacchi, Neuropharmacology *20*, 845 (1981).
13 J.D. Doherty, M. Simonovic, R. So and H.Y. Meltzer, Eur. J. Pharmac. *65*, 139 (1980).
14 K.M. Johnson and K.C. Oeffinger, Life Sci. *28*, 361 (1981).
15 S.H. Snyder, Nature *285*, 355 (1980).
16 R.S. Aronstam, Life Sci. *28*, 59 (1981).

Drug modification of silver-induced sodium transport across toad skin[1]

G.A. Gerencser, S.Y. Loo and K.M. Cornette

Department of Physiology, College of Medicine, University of Florida, Gainesville (Florida 32610, USA), and Department of Physiology, School of Medicine, University of Hawaii, Honolulu (Hawaii 96822, USA), 27 November 1981

Summary. Stimulation of active Na^+ transport in the toad skin by antidiuretic hormone (ADH) and p-chloromecuribenzoate (P-CMB) was blunted by the presence of silver (Ag^+). Amiloride inhibited active Na^+ transport, equivalently, whether Ag^+ was present or not.

Silver ions (Ag^+) have been shown to stimulate electrical characteristics and ion transport across various epithelial preparations[2,3]. In fact, Walser[4] reported a stimulatory effect on short-circuit current (SCC) by Ag-AgCl electrodes immersed directly into the bathing solutions of isolated toad bladder. Curran concluded from studies of Ag^+ effects on permeability properties of frog skin that Ag^+ may increase skin shunt permeability and may affect the cation selectivity of the outer membrane, possibly by reacting with sulfhydryl groups. Gerencser et al.[6] suggested that silver chloride, or their complexes, bind to specific membrane groups in enhancing active sodium absorption across toad skin. The present work was therefore undertaken in order to assess possible mechanisms of silver-induced sodium transport across toad skin.

Materials and methods. Adult toads, *Bufo marinus*, of either sex were kept fasting at room temperature of 25 °C prior to experimentation. The transmural potential difference and SCC were measured across sheets of toad skin similar to those methods employed by Schultz and Zalusky[7] except that both Ag-AgCl and agar electrolytic bridges were used to apply external current to the system. These 2 methods were compared with one another and differences between them were analyzed statistically using the Student's t-test. The silver concentration of the bathing medium was determined using an atomic absorption spectrophotometer as described previously[6]. The skin was aerated and mounted between identical phosphate-buffered NaCl Ringer solution of the type described by Adrian[8].

Results. Using toad skin preparations from the same ani-