Biochemical Pharmacology, Vol. 29, pp. 857–868

0006-2952/80/0315-0857 $02.00/0

CHARACTERIZATION AND LOCALIZATION OF CATECHOLAMINE-SUSCEPTIBLE Na–K ATPase ACTIVITY OF RAT STRIATUM: STUDIES USING CATECHOLAMINE RECEPTOR (ANT)AGONISTS AND LESION TECHNIQUES

JACQUES A. VAN DER KROGT and RENÉ D. M. BELFROID
Leiden University Medical Centre, Department of Pharmacology, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands

(*Received* 24 *July* 1979; *accepted* 26 *October* 1979)

Abstract—Na–K ATPase activity of homogenates of rat striatum is stimulated by dopamine (DA) and noradrenalin (NA) in similar and concentration-dependent ways. The enzyme is also stimulated by apomorphine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN), agonists of DA receptors. Other DA agonists, such as bromocriptine and piribedil, show no effect at all, while another, lergotrile, stimulates only slightly. The stimulatory effect of DA and apomorphine is not counteracted by haloperidol. *cis*-Flupenthixol, another DA receptor antagonist, does strongly inhibit DA stimulation of striatal Na–K ATPase activity at concentrations which inhibit basal activity to a limited extent only. However, this effect is not related to the DA receptor blocking activity of the drug, as *trans*-flupenthixol, an isomer which is much less active in DA receptor blocking tests, inhibits the stimulation by DA even more. With drugs blocking α and β noradrenergic receptors (phentolamine and dihydroergotoxine, respectively propranolol), no specific inhibition of the NA stimulation of Na–K ATPase activity of striatum, cerebral cortex and cerebellum is found. In addition, no effect of the α receptor agonist clonidine is observed. From these experiments with catecholamine receptor agonists and antagonists, we conclude that stimulation of brain Na–K ATPase most probably is not related to catecholamine receptor mechanisms. Lesion experiments suggest that the DA susceptible striatal Na–K ATPase activity is not specifically localized in DA neurons or in DA innervated neurons. Neither degeneration of DA-containing terminals (caused by 6-hydroxydopamine) nor degeneration of neurons postsynaptically with regard to DA terminals (caused by kainic acid) affects DA susceptibility of the striatal enzyme. We therefore conclude that catecholamine susceptibility probably is a general characteristic of striatal Na–K ATPase (and probably also of that of other brain regions). Our conclusions are in line with suggestions in the literature that stimulation of brain Na–K ATPase by catecholamines is a nonspecific phenomenon and is indirectly effected by abolition of the inhibitory effect of divalent metal ions on the enzyme. Indeed, the striatal enzyme is stimulated by EDTA too.

It is widely believed that Na–K ATPase (Na^+–K^+-activated, Mg^{2+}-dependent ATP phosphohydrolase, EC 3.6.1.3) plays a crucial part in the functioning of neurons. The enzyme is thought to be responsible for the maintenance and re-establishment of the cation gradient across the neuronal membrane which is essential for impulse propagation [1]. In addition, several data suggest a role of the enzyme in the regulation of release and uptake of neurotransmitters [2–4]. Since 1972 numerous investigators have reported that Na–K ATPase activity of a variety of brain tissue preparations can be stimulated *in vitro* by catecholamines [5–17]. The mechanism of this phenomenon is unclear. In some cases reduction of the stimulatory effect of catecholamines by catecholamine (CA) receptor antagonists is described, suggesting a receptor-mediated process [9, 17–20]. Other investigators are of the opinion that catecholamines do not stimulate Na–K ATPase directly, but rather disinhibit the enzyme by chelating inhibitory metal ions [8, 15, 21].

In view of studies on dopaminergic transmission [22], we were particularly interested in the effect of dopamine (DA) on Na–K ATPase activity of striatal preparations. On this point the literature presented very few data. At the start of our investigations there only was a paper by Logan and O'Donovan [12], reporting absence of an effect of DA on Na–K ATPase activity of a striatal synaptic membrane preparation of rabbits. In the course of our studies, however, two groups reported stimulation by DA of the enzyme in striatal homogenates, viz. Sulakhe *et al.* (rat and rabbit [16]) and Akagawa and Tsukada (rat [17]). Our results as described in this paper confirm this observation of the latter groups. To further characterize the effect of DA on striatal Na–K ATPase, we studied the effect of a number of drugs with well-known stimulating or blocking actions on DA and noradrenalin (NA) receptors. For comparison, we performed parallel assays on other brain area preparations. We also investigated the effect of lesions on the DA stimulation of the striatal enzyme in order to see whether the DA susceptible enzyme has a specific localization. Some of our findings, presented in this paper, have been reported at the Second Meeting of the European Society for Neurochemistry, Göttingen, August 1978 [23].

MATERIALS AND METHODS

Chemicals. ATP, isolated from equine muscle, and synthetic ATP, both disodium salts, were obtained from Sigma. The sources of the drugs were: ouabain: Merck; dopamine HCl: Sigma; *l*-noradrenalin bitartrate: Serva; serotonin creatininsulfate: Merck; apomorphine HCl: Sandoz; α-bromocriptine mesylate: Sandoz; lergotrile mesylate: Eli Lilly; piribedil mesylate: Servier; 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene HBr: Dr. B. H. C. Westerink, Laboratory for Pharmaceutical and Analytical Chemistry, Groningen, The Netherlands; clonidine HCl: Boehringer Ingelheim; haloperidol: Janssen; *cis*- and *trans*-flupenthixol 2HCl: Lundbeck; phentolamine HCl: Ciba-Geigy; propranolol HCl: ICI; dihydroergotoxine mesylate: Sandoz; 6-hydroxydopamine HCl: Aldrich; kainic acid: Sigma. All other chemicals were p.a. quality.

Brain homogenates. Male rats of the laboratory-bred SPF Wistar strain, weighing 200–230 g, were decapitated. Brain dissections were performed on an ice-cooled glass plate. Brain tissue was homogenized in 50–125 vol. of ice-cold deionized water, using a glass Potter–Elvehjem homogenizer with teflon pestle.

Brain lesions. All lesions were unilaterally made under ether anaesthesia. 6-Hydroxydopamine (6-OHDA) lesions were made by implanting a pellet of about 25 μg 6-OHDA into the right nigro-striatal tract (coordinates according to König and Klippel [24] in mm: $A = 4.0$; $L = 1.5$; $V = -2.6$) by means of a cannula as described by Smelik [25]. After about 18 days the rats were killed. Homogenates of the striata were analysed for ATPase activities, for DA content (method of Westerink and Korf [26]) and for protein content (method of Lowry *et al.* [27]). Lesions which caused more than 90 per cent reduction of DA in comparison with the non-lesioned striatum were considered as successful and only results of these lesions are presented (8 out of 9).

Kainic acid lesions were made by injecting 1.25 or 2.25 μg kainic acid in 1 μl isotonic saline into the right striatum over a 5 min period (coordinates: $A = 7.6$; $L = 3.0$; $V = 0.4$). After injection the cannula was left in place for another 5 min. Vehicle (1 μl) was injected into the left striatum. The animals were killed 8 days after the injection. Homogenates of the striata were analysed for ATPase activities as well as for protein content.

Na–K *ATPase assay.* Na–K ATPase activity was taken as the difference between ATPase activity measured in the presence of Mg^{2+}, Na^+ and K^+ (total ATPase activity) and ATPase activity measured in the presence of Mg^{2+}, Na^+ and ouabain (Mg ATPase activity).

Total ATPase activity was assayed in a medium containing 4 mM ATP (disodium salt), 100 mM NaCl, 20 mM KCl, 6 mM $MgCl_2$, 10 mM Tris-maleate and homogenate of 0.4–0.5 mg brain tissue. For measurement of Mg ATPase activity, KCl was omitted and 1 mM ouabain was added. The final volume was 1.0 ml, the final pH 7.3 at 37°. The assay medium minus ATP was preincubated at 37° for 10 min. The reaction was started by addition of ATP and after 20 min terminated with 0.5 ml of cold 7.5% (w/v) trichloroacetic acid. After cooling for some minutes, 1.5 ml of colour reagent (1.15% ammonium heptamolybdate in 1.32 N sulfuric acid, in which 92 mg/ml ferrous sulfate was dissolved immediately before use [28]) was added, followed by centrifugation in the cold for 5 min at 3000 r.p.m. The extinction of the supernatant was read at 700 nm 30 min after addition of the colour reagent.

For measurement of tissue-blank values, homogenate was added after trichloroacetic acid. All ATPase assays were performed in triplicate. ATPase activities are expressed as μmoles Pi released per g tissue or (for lesion experiments) per mg protein and per hr incubation at 37°.

The drugs used in our study were added to the preincubation media. Fresh solutions were prepared immediately before use. The drugs were carefully checked for possible effects on the final pH of the assay media. To dissolve haloperidol, acetic acid was used. The pH of this solution was adjusted with Tris to a limited extent to avoid precipitation. Using 10^{-4} M haloperidol, the final pH of the ATPase assay media was maximally 7.1. Bromocriptine, too, needed some acetic acid in order to dissolve. To correct the (small) vehicle effects in these experiments, a corresponding volume of a similarly prepared vehicle was added to all control assay media.

Presentation of results and statistical evaluation. In order to compensate for variability in the levels of the ATPase activities owing to the use of different ATP preparations (see Results) and to day-to-day variations, we calculated our results (and presented them in tables and figures) as percentages of paired basal or control activities. The absolute values of the latter, however, are mentioned in the legends to the figures and in the tables. Since we were primarily interested in effects on Na–K ATPase activity, results of Mg ATPase assays are not always shown, but are sometimes only mentioned in the legends.

Statistical evaluation was done by Student's *t*-test for paired data. A $P < 0.05$ was chosen as the level of significance.

RESULTS

(1) *Effect of DA and other biogenic amines on* Na, K-*ATPase activity of striatum and other brain regions*

Effect of DA. As is shown in the upper part of Fig. 1, Na–K ATPase activity of striatal homogenates is stimulated by DA in a concentration-dependent way, with a maximum at 10^{-4} M. The lower part of Fig. 1 presents, under the same circumstances, the activities of Mg ATPase, an enzyme distinct from Na–K ATPase but in crude enzyme preparations always accompanying it. Its activity is also stimulated by DA, but to a far less extent, e.g. 10^{-4} M DA more than doubles Na–K ATPase activity, but enhances Mg ATPase activity by only 20 per cent. The columns A, B and C of Fig. 1 indicate results obtained using different ATP preparations as substrate, viz. ATP isolated from equine muscle and containing an inhibitor of Na–K ATPase, recently identified as vanadate (see Discussion), equine muscle ATP essentially free of vanadate and synthetic ATP, respectively. Using these different ATP prep-

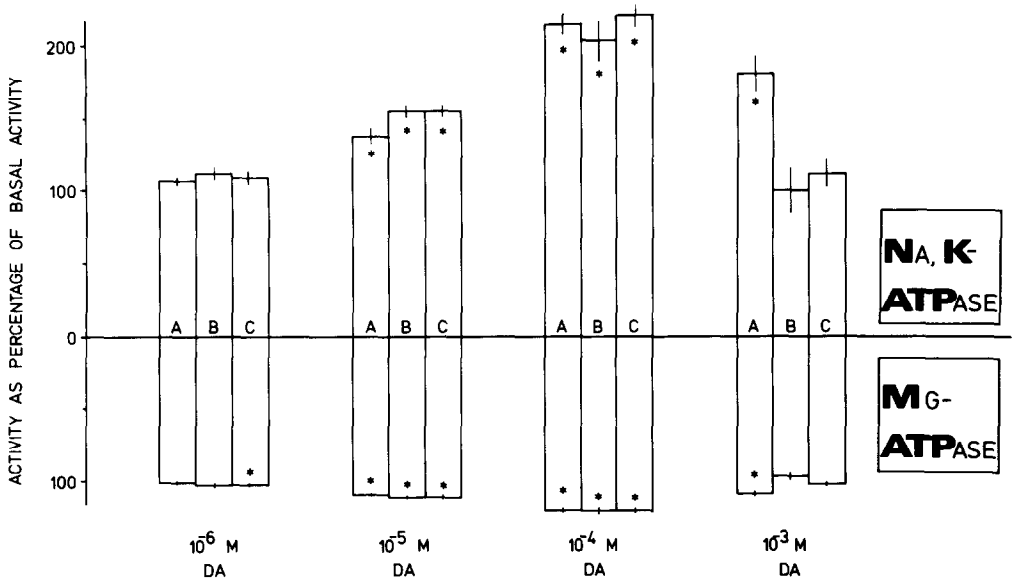

Fig. 1. Effect of various concentrations of DA on Na–K ATPase (upper part of the figure) and Mg ATPase activity (lower part) of striatal homogenates, using three different ATP preparations as substrate, viz. vanadate-containing equine muscle ATP (A), vanadate-free equine muscle ATP (B) and synthetic ATP (C). Results shown are means of 9 (A), 4 (B) and 8 (C) experiments ± S.E.M. ATPase activities are expressed as percentages of the paired basal activities. Activities significantly different from basal activities are indicated by *. Basal activities (in μmoles Pi g^{-1} hr^{-1}) : A: Na–K ATPase: 532 ± 51; Mg ATPase: 1215 ± 14; B: Na–K ATPase: 895 ± 34; Mg ATPase: 1193 ± 21; C: Na–K ATPase: 715 ± 41; Mg ATPase: 1258 ± 15.

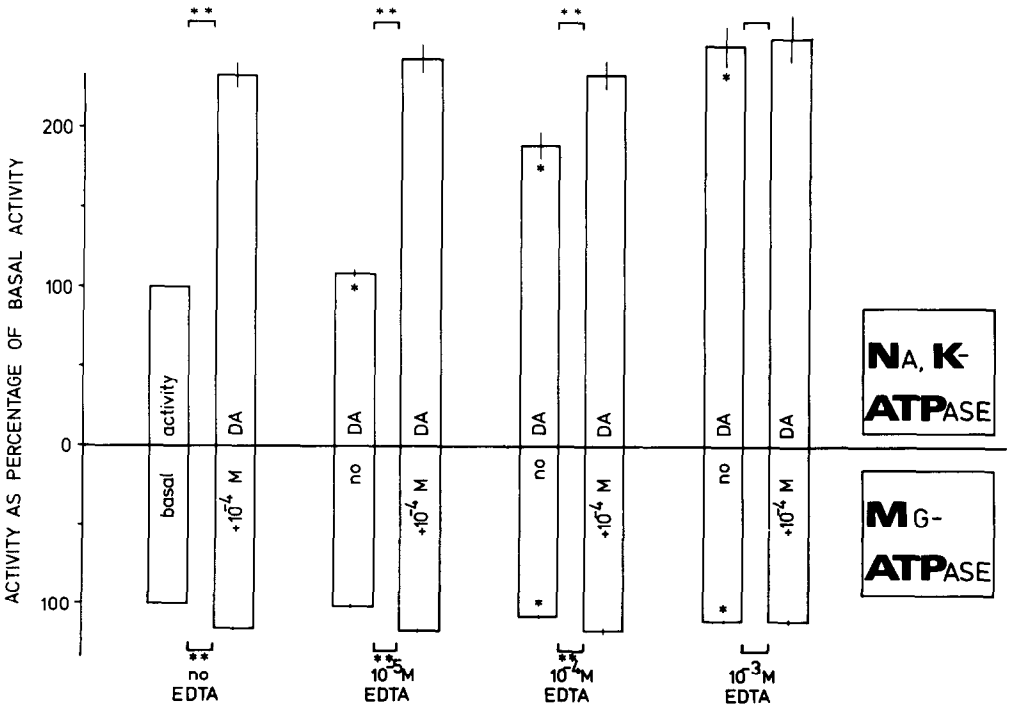

Fig. 2. Effect of 10^{-4} M DA on striatal Na–K ATPase (upper part of the figure) and Mg ATPase activity (lower part) in the presence of various concentrations of EDTA. Results shown are means of 3 experiments ± S.E.M. ATPase activities are expressed as percentages of the paired basal activities. Statistically significant effects of EDTA compared with the basal activities are indicated by *, those of 10^{-4} M DA compared with the corresponding control activities by **. Basal activities (in μmoles Pi g^{-1} hr^{-1}): Na–K ATPase: 625 ± 22; Mg ATPase: 1523 ± 16.

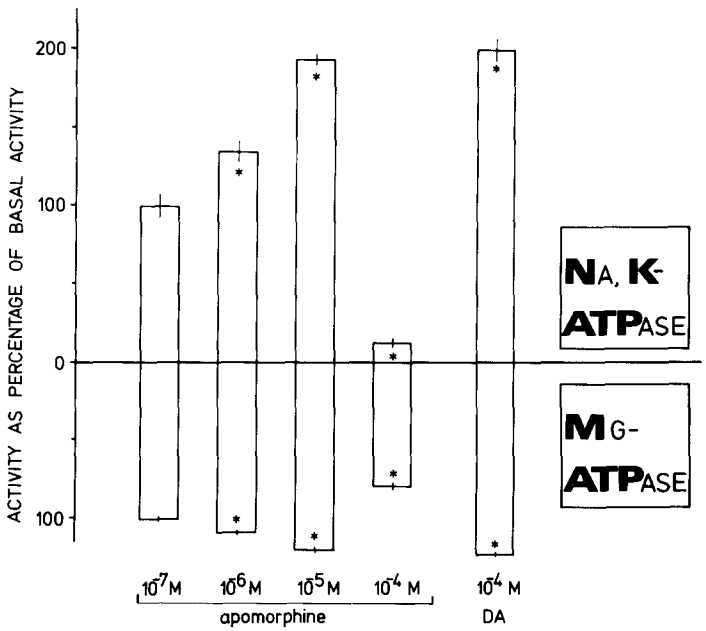

Fig. 3. Effect of various concentrations of apomorphine on striatal Na–K ATPase (upper part of the figure) and Mg ATPase activity (lower part), compared with the effect of 10^{-4} M DA. Results shown are means of 6 experiments ± S.E.M. ATPase activities are expressed as percentages of the paired basal activities. Activities significantly different from basal activities are indicated by *. Basal activities (in μmoles Pi g^{-1} hr^{-1}): Na–K ATPase: 815 ± 120; Mg ATPase: 1195 ± 48.

arations, only minor differences in the stimulative effect of DA on Na–K ATPase are observed, the most notable being that 10^{-3} M DA only stimulates the enzyme when assayed with vanadate-containing ATP. However, we do not attach much importance to this difference, as in other experiments (not shown here) we found that the effect of such high CA concentrations vary considerably. As can be seen in the legend to Fig. 1, absolute Na–K ATPase activities are higher with the vanadate-free ATP preparations than with the vanadate-containing one, which fits in an inhibitory effect of vanadate. The Mg ATPase activities show no differences. A great many of our experiments had been completed before the vanadate-free equine muscle ATP preparation became obtainable. As the comparative experiments described above showed only a negligible effect of the vanadate impurity on the stimulation by the most extensively studied DA concentrations (10^{-5} and 10^{-4} M), we decided not to repeat all our previous experiments; those which were repeated showed similar results. Yet, we continued our studies with a vanadate-free preparation of equine muscle ATP as soon as it was obtainable.

Comparative experiments with homogenates of striatum, cerebral cortex and cerebellum showed that the Na–K ATPase activities of these regions are stimulated by DA to a similar extent (results not shown).

Effect of NA and serotonin (5HT). NA and 5HT are also capable of increasing the activity of striatal Na–K ATPase in a concentration-dependent manner. NA has the same potency as DA, that of 5HT is far less: 10^{-4} M of the catecholamines doubles enzyme activity, whereas 10^{-4} M 5HT stimulates the enzyme by about 30 per cent (results not shown).

Effect of DA in the presence of EDTA. EDTA causes a concentration-dependent stimulation of striatal Na–K ATPase (and Mg ATPase) activity (Fig. 2). Stimulation by 10^{-4} M DA appears to be only partly additive with this effect of EDTA: in all cases about the same maximal activity is reached.

(2) *Effect of CA receptor agonists and antagonists on basal and stimulated* Na–K *ATPase activity*

Effect of DA receptor agonists. As can be seen in Fig. 3, the DA receptor agonist apomorphine induces at lower concentrations (10^{-6} and 10^{-5} M) a concentration-dependent increase of striatal Na–K ATPase activity, whereas 10^{-4} M almost completely inhibits the enzyme. The latter concentration also inhibits Mg ATPase activity, though to a lesser extent. Apomorphine appears to be more potent than DA: the stimulation by 10^{-5} M apomorphine practically corresponds with that caused by 10^{-4} M DA. Apomorphine not only stimulates Na–K ATPase activity of striatal homogenates, but also that of other brain regions (results not shown).

In addition to apomorphine we have studied the effect of some other drugs known as more or less specific DA receptor agonists, viz. bromocriptine, lergotrile, piribedil and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN). Results of experiments with the three former drugs are reported in Table 1; the ADTN results are shown in Fig. 4. In contrast to apomorphine, bromocriptine and piribedil have no effect whatsoever on striatal Na–K ATPase activity. Lergotrile does stimulate the

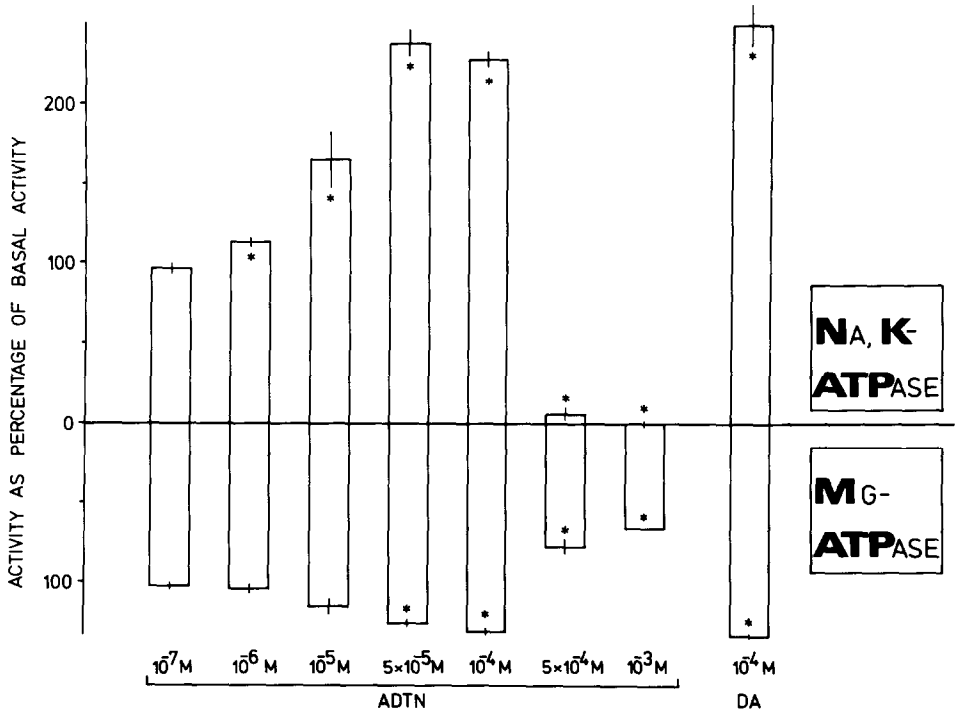

Fig. 4. Effect of various concentrations of ADTN on striatal Na–K ATPase (upper part of the figure) and Mg ATPase activity (lower part), compared with the effect of 10^{-4} M DA. Results shown are means of 4 experiments (except 5×10^{-5} and 5×10^{-4} M: 3 experiments) ± S.E.M. ATPase activities are expressed as percentages of the paired basal activities. Activities significantly different from basal activities are indicated by *. Basal activities (in μmoles Pi g^{-1} hr^{-1}): Na–K ATPase: 1002 ± 34; Mg ATPase: 1384 ± 21.

enzyme, but far less than apomorphine: a 10^{-5} M concentration of the latter compound increases Na–K ATPase activity by about 100 per cent, whereas the effect of a ten times higher concentration of lergotrile amounts to less than 30 per cent. The drugs mentioned in Table 1 could not be studied at higher concentrations than the ones shown, because at 5 (bromocriptine and lergotrile) resp. 2 (piribedil) times higher concentration, precipitation occurred in the test tubes. From Fig. 4 it can be inferred that the rigid DA analogue ADTN stimulates striatal Na–K ATPase as effectively as DA itself: both compounds more than double enzyme activity at 10^{-4} M. However, a remarkable difference between ADTN and DA emerges at 10^{-3} M: at this concentration the effect of DA varies between stimulation and no effect (see Fig. 1), whereas ADTN completely inhibits Na–K ATPase activity. In this respect ADTN resembles apomorphine, although inhibition by the latter drug already takes place at a lower concentration.

Effect of NA receptor agonist. The α NA receptor

Table 1. Effect of bromocriptine, lergotrile and piribedil on striatal Na–K ATPase activity, compared with the effect of 10^{-4} M DA

	Bromocriptine	Lergotrile	Piribedil
No drug	100% (765 ± 84 μmoles)	100% (1040 ± 62 μmoles)	100% (1105 ± 41 μmoles)
10^{-6} M	107.7 ± 9.5%	105.6 ± 4.5%	97.9 ± 1.8%
10^{-5} M	102.2 ± 6.1%	108.9 ± 2.7%*	103.7 ± 1.7%
5×10^{-5} M			96.5 ± 3.9%
10^{-4} M		128.5 ± 4.6%*	
10^{-4} M DA	210.5 ± 11.4%*	222.9 ± 13.2%*	207.9 ± 8.8%*

Values given are means of 4 (bromocriptine), 6 (lergotrile) and 3 experiments (piribedil) ± S.E.M. Na–K ATPase activities are expressed as percentages of the paired basal activities. The latter, expressed in μmoles Pi g^{-1} hr^{-1}, are given in brackets. Activities significantly different from basal activities are indicated by *. None of the drugs (except DA) significantly affected Mg ATPase activity (results not shown).

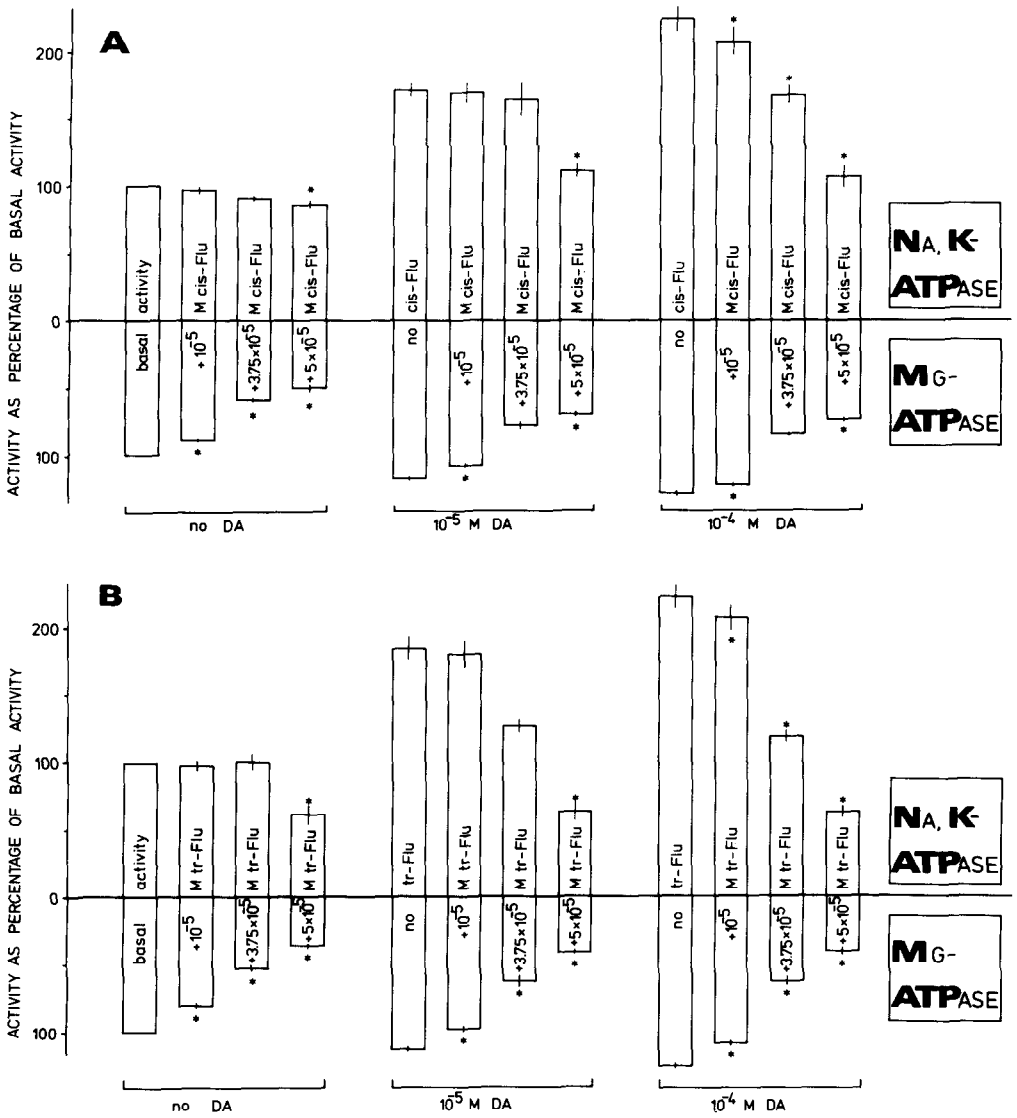

Fig. 5. Effect of various concentrations of *cis*-flupenthixol (A) and *trans*-flupenthixol (B) on basal and DA stimulated striatal Na–K ATPase (upper part of the figure) and Mg ATPase activity (lower part). Results shown are means of 6 experiments ± S.E.M. (except 3.75×10^{-5} M: 2 experiments ± range). ATPase activities are expressed as percentages of the paired basal activities. Activities significantly different from the corresponding control activities are indicated by *. Basal activities (in μmoles Pi $g^{-1} hr^{-1}$): A: Na–K ATPase: 978 ± 47; Mg ATPase: 1312 ± 31; B: Na–K ATPase: 899 ± 36; Mg ATPase: 1377 ± 32.

agonist clonidine, in a concentration range of 10^{-6} to 10^{-3} M, does not significantly change striatal Na–K ATPase activity. At 10^{-3} M there is a tendency to inhibition (results not shown).

Effect of DA receptor antagonists. A 10^{-4} M concentration of the DA receptor blocker haloperidol does not affect stimulation of striatal Na–K ATPase activity by 10^{-5} or 10^{-4} M DA (see Table 2), nor does haloperidol influence the stimulatory effect of NA and apomorphine (results not shown). Haloperidol inhibits striatal Mg ATPase, but the basal and DA stimulated activities of the enzyme to the same extent. In contrast with haloperidol, flupenthixol does inhibit DA stimulation of striatal Na–K ATPase activity. This is illustrated in Fig. 5: panel A shows the effect of the *cis* isomer, panel B that of the *trans* isomer of flupenthixol. Also basal Na–K ATPase activity is inhibited by both isomers. However, the effects on the stimulated Na–K ATPase activities are much stronger: 5×10^{-5} M *cis*-flupenthixol very markedly reduces the increase of enzyme activity by 10^{-5} and 10^{-4} M DA, while 5×10^{-5} M *trans*-flupenthixol even completely eliminates the DA activation of striatal Na–K ATPase activity. Also striatal Mg ATPase is inhibited by the flupenthixol isomers, but, as in the case of haloperidol, basal and stimulated activities to about the same extent.

Effect of NA receptor antagonists. Table 3 shows the effects of the α blockers phentolamine and

Table 2. Effect of haloperidol (HAL) on basal and DA stimulated striatal Na–K ATPase and Mg ATPase activity

	No DA	10^{-5} M DA	10^{-4} M DA
Na–K ATPase			
No HAL	100% (600 ± 63 μmoles)	100% (1103 ± 195 μmoles)	100% (1668 ± 213 μmoles)
10^{-4} M HAL	97.4 ± 5.7%	95.2 ± 4.2%	98.0 ± 2.2%
Mg ATPase			
No HAL	100% (1269 ± 44 μmoles)	100% (1372 ± 42 μmoles)	100% (1479 ± 55 moles)
10^{-4} M HAL	68.4 ± 3.0%*	73.6 ± 2.2%*	79.3 ± 1.4%*

Values given are means of between 8 and 10 experiments ± S.E.M. ATPase activities are expressed as percentages of the corresponding paired control activities in the absence of haloperidol. The latter activities, expressed in μmoles $Pi\,g^{-1}\,hr^{-1}$, are given in brackets. Activities significantly different from the corresponding control activities are indicated by *. The ATPase activities given in this table were measured at a final pH of the assay medium of 7.1 (see Materials and Methods).

dihydroergotoxine and of the β blocker propranolol on the stimulation by 10^{-5} and 10^{-4} M NA of Na–K ATPase activity of homogenates of striatum, cerebral cortex and cerebellum.

Phentolamine, in a concentration of 10^{-4} M, stimulates basal Na–K ATPase activity of all three brain regions. If no correction is made for this increase of the basal activity, phentolamine does not show any effect on the stimulation of the Na–K ATPase activities by NA. But if this correction is made, a small and in most cases significant inhibition of the stimulatory effect of NA is observed (data not shown). However, it is uncertain whether the correction is allowed, since phentolamine stimulation perhaps only occurs under control conditions. We have also studied the effect of 5×10^{-4} M phentolamine, but at this concentration very inconsistent results were obtained.

After dihydroergotoxine, in a concentration of 10^{-4} M, no significant change in the Na–K ATPase activities of striatum and cerebellum was observed, either in the basal activities, or in the NA stimulation. The drug does cause a statistically significant inhibition of the stimulated Na–K ATPase activity of

Table 3. Effect of phentolamine (PHEN), dihydroergotoxine (DHE) and propranolol (PROP) on basal and NA stimulated Na–K ATPase activity of homogenates of striatum, cerebral cortex and cerebellum

	No NA	10^{-5} M NA	10^{-4} M NA	N
Striatum				
No antagonist	100%	100%	100%	
PHEN 10^{-4} M	117.6 ± 2.9%*	104.8 ± 5.0%	98.9 ± 3.9%	8
DHE 10^{-4} M	99.5 ± 6.1%	110.8 ± 3.8%	112.9 ± 3.7%	3
PROP 10^{-4} M	90.1 ± 6.0%	110.4 ± 8.2%	105.1 ± 1.6%*	3
10^{-3} M	64.0 ± 5.0%*	78.9 ± 2.5%*	72.7 ± 5.5%*	3
Cerebral cortex				
No antagonist	100%	100%	100%	
PHEN 10^{-4} M	125.2 ± 5.5%*	105.7 ± 3.5%	94.6 ± 4.2%	8
DHE 10^{-4} M	89.7 ± 2.9%*	95.4 ± 0.8%*	88.1 ± 1.4%*	4
PROP 10^{-4} M	106.0 ± 12.4%	95.8 ± 3.8%	97.2 ± 2.1%	3
10^{-3} M	38.2 ± 12.1%*	45.9 ± 1.9%*	39.3 ± 0.9%*	3
Cerebellum				
No antagonist	100%	100%	100%	
PHEN 10^{-4} M	119.5 ± 7.3%*	99.6 ± 4.9%	97.8 ± 5.8%	10
DHE 10^{-4} M	94.1 ± 2.7%	99.1 ± 3.3%	97.7 ± 2.6%	8
PROP 10^{-4} M	91.0 ± 3.6%	89.0 ± 1.0%*	86.5 ± 7.1%	3
10^{-3} M	43.6 ± 10.3%*	44.8 ± 5.7%*	44.8 ± 2.0%*	3

Values given are means of N experiments ± S.E.M. Na–K ATPase activities are expressed as percentages of the corresponding paired control activities in the absence of an antagonist. The values of the latter activities are not given, as the results of various experiments are combined in this table. Activities significantly different from the corresponding control activities are indicated by *. Phentolamine has no effect on Mg ATPase activities. 10^{-4} M dihydroergotoxine causes significant inhibition of Mg ATPase activity, ranging from 7 per cent (cerebellum) to 18 per cent (striatum and cortex). 10^{-4} M propranolol does not affect Mg ATPase activities; 10^{-3} M propranolol, however, significantly inhibits the enzyme of all three brain regions by about 30 per cent (results not shown).

Table 4. Effect of implantation of 6-OHDA into the nigrostriatal tract on the basal and DA stimulated striatal ATPase activities (a) and on the striatal DA content (b)

(a) ATPase activities

	No DA	10^{-5} M DA	10^{-4} M DA
Na–K ATPase			
Control side	100% (5.57 ± 0.38 μmoles)	100% (7.78 ± 0.87 μmoles)	100% (14.26 ± 0.95 μmoles)
Lesion side	102.2 ± 6.0%	103.4 ± 6.9%	97.2 ± 3.9%
Mg ATPase			
Control side	100% (10.50 ± 0.45 μmoles)	100% (11.32 ± 0.58 μmoles)	100% (12.51 ± 0.69 μmoles)
Lesion side	102.7 ± 2.2%	105.0 ± 3.3%	103.3 ± 3.8%

(b) DA content

	μg DA/g tissue	% of control side
Control side	9.25 ± 0.88	100
Lesion side	0.15 ± 0.12	1.3 ± 0.9

Values given are means of 8 experiments ± S.E.M. ATPase activities are expressed as percentages of the corresponding paired control activities. The latter activities, expressed in μmoles P_i mg^{-1} protein hr^{-1}, are given in brackets. Protein content of the homogenates: control side: 112.8 ± 1.4 mg/g; lesion side: 109.4 ± 2.0 mg/g. The only significant lesion effect is that on striatal DA content.

cerebral cortex; however, this effect is not specific for the stimulated activity, as the basal activity is inhibited to about the same extent. A higher concentration of dihydroergotoxine could not be studied because of precipitation of the drug in the test tubes. Mg ATPase activity of all brain regions is significantly inhibited by dihydroergotoxine.

Propranolol, in a concentration of 10^{-3} M, significantly inhibits the Na–K ATPase activities of all three brain regions. No specific inhibition of the stimulative effect of NA is found, however, as the enzyme activities in the presence of NA are decreased by about the same (or a smaller) percentage as the basal activities. Similar results have been found regarding the propranolol effect on the DA stimulated Na–K ATPase activity (results not shown). A 10^{-3} M propranolol concentration also markedly inhibits the Mg ATPase activities. Lower propranolol concentrations did not cause a specific inhibition of the NA stimulated Na–K ATPase activities either.

(3) *Effect of lesions on striatal* Na–K *ATPase activity and its stimulation by DA*

Effect of implantation of 6-hydroxydopamine (6-OHDA) into the nigrostriatal tract. From the nearly complete disappearance of DA from the striatum 18 days after 6-OHDA implantation (see Table 4, part B), one can infer that at that time a very extensive degeneration of striatal dopaminergic terminals has taken place. In other experiments (to be published) we have found that such an extensive degeneration is accompanied with an almost complete disappearance of striatal tyrosine hydroxylase activity. However, as part A of Table 4 shows, the lesion did not affect either the basal, or the DA stimulated striatal ATPase activities. Absence of an effect on the basal ATPase activities, in spite of the disappearance of nearly all DA nerve terminals, is not surprising, as the DA terminals comprise only a small fraction of the total population of striatal nerve terminals [29], not to mention total striatal tissue mass. Moreover, degeneration of neurons is usually followed by proliferation of glial tissue, which also possesses Na–K and Mg ATPase activity [30].

Effect of injection of kainic acid into the striatum. Eight days after intrastriatal injection of 2.25 μg kainic acid, basal Na–K ATPase activity is significantly reduced by about 25 per cent. Since, however, the DA stimulated activities are reduced by about the same percentage, the DA susceptibility of the enzyme left is similar to that of the enzyme of the sham-lesioned striatum (Table 5). So kainic acid causes disappearance of Na–K ATPase activity, but the enzyme that disappeared was not preferentially stimulated by DA (nor was the contrary the case). In contrast to Na–K ATPase, striatal Mg ATPase activity is not reduced after kainic acid injection; there even is a small (but significant) increase. The latter can only partly be explained by the somewhat lower protein content of the homogenates of the lesioned striatum (for lesion experiments, activities are calculated per mg protein). At the moment we have no explanation for the differential effect of kainic acid on the ATPase activities. As Table 5 shows, 1.25 μg kainic acid did not yet change the ATPase activities significantly.

DISCUSSION

We found that Na–K ATPase activity of striatal homogenates is appreciably stimulated by 10^{-5} M DA and is more than doubled by 10^{-4} M DA. Stimulation of the striatal enzyme, however, is not a specific property of DA, as also NA and 5HT bring about activation, NA with about the same potency as DA, but 5HT to a much smaller extent. So it is clear that the susceptibility of striatal Na–K ATPase

Table 5. Effect of intrastriatal injection of 1.25 (a) or 2.25 (b) μg kainic acid on basal and DA stimulated striatal ATPase activities

	No DA	10^{-5} M DA	10^{-4}M DA
(a) 1.25 μg kainic acid			
Na–K ATPase			
Control side	100%	100%	100%
Lesion side	(6.54 ± 0.28 μmoles)	(10.06 ± 0.22 μmoles)	(15.03 ± 0.43 μmoles
	87.6 ± 10.9%	91.1 ± 6.7%	87.1 ± 8.9%
Mg ATPase			
Control side	100%	100%	100%
	(12.23 ± 0.14 μmoles)	(13.24 ± 0.18 μmoles)	(14.39 ± 0.17 μmoles)
Lesion side	108.1 ± 3.5%	107.4 ± 2.5%*	105.3 ± 1.4%*
(b) 2.25 μg kainic acid			
Na–K ATPase			
Control side	100%	100%	100%
	(5.04 ± 0.36 μmoles)	(8.20 ± 0.66 μmoles)	(12.26 ± 0.73 μmoles)
Lesion side	77.7 ± 6.3%*	69.2 ± 7.6%*	75.1 ± 8.3%*
Mg ATPase			
Control side	100%	100%	100%
	(11.01 ± 0.77 μmoles)	(11.67 ± 0.91 μmoles)	(12.67 ± 0.28 μmoles)
Lesion side	112.2 ± 3.6%*	113.4 ± 3.9%*	109.9 ± 3.2%*

Values given are means of 7–8 experiments ± S.E.M. ATPase activities are expressed as percentages of the corresponding paired activities of the control side.These control side activities, expressed in μmoles P_i mg^{-1} protein hr^{-1}, are given in brackets. Protein content of the homogenates (in mg/g): (a) control side: 105.2 ± 1.4; lesion side: 103.6 ± 2.2; (b) control side: 106.4 ± 4.1; lesion side: 99.8 ± 4.5. Lesion side activities significantly different from corresponding control side activities are indicated by *.

for stimulation by the catecholamines bears no relation to their individual striatal concentrations. The similar DA effect on Na–K ATPase activities of different brain regions with widely divergent CA contents point to the absence of a relation between CA susceptibility and total CA contents too. In contrast with our findings, Akagawa and Tsukada [17] found that NA enhanced striatal Na–K ATPase activity with only half the potency of DA. Their DA effect is comparable with ours. In addition to the effect on Na–K ATPase, catecholamines also stimulate Mg ATPase activity, although to a much smaller extent. This has also been reported by various other authors [5–8, 11, 18].

During our investigations a number of reports were published claiming the presence of an inhibitor of Na–K ATPase in the Sigma ATP preparation isolated from equine muscle, which we used for our assays [31, 32]. Recently this inhibitor was identified as vanadate [33, 34]. Various authors demonstrated that the effect of this inhibitor was abolished by catecholamines [31–35]. Therefore we had to exclude the possibility that the stimulatory effect of catecholamines on brain Na–K ATPase activity as observed in our experiments was due to the vanadate present in our ATP preparation. For this purpose some comparative experiments with vanadate-free ATP preparations were performed. It was found that presence of contaminant vanadate had no substantial influence on the stimulation of striatal Na–K ATPase activity by DA, except perhaps in the case of very high DA concentrations.

In order to gain further insight into the mechanism of the stimulatory effect of DA on striatal Na–K ATPase activity and its relation to receptor mechanisms, we investigated the effect of some drugs with known actions on DA receptors. The DA receptor agonist apomorphine [36], the first drug we studied, strongly stimulates striatal Na–K ATPase activity and in this respect it is even more potent than DA: the enzyme activity is more than doubled by 10^{-5} M apomorphine. Further increase of the apomorphine concentration by a factor of 10 changes the effect into almost complete inhibition. Using a similar concentration range as we did, Schaefer *et al.* [37] observed only inhibition of Na–K ATPase activity (of whole brain) by apomorphine. The difference in origin of the brain preparations cannot explain the discrepancy, as we found stimulation by apomorphine also with homogenates of other brain regions. A more likely cause are differences in treatment of the brain preparations studied: Schaefer *et al.* used purified brain preparations, we used crude homogenates.

The fact that striatal Na–K ATPase activity in our hands can be stimulated by DA as well as by apomorphine points to similarity in stimulation characteristics between striatal Na–K ATPase and DA receptors. An obvious way to verify this idea is to investigate the effect of a receptor blocking drug. For this purpose we used haloperidol [38]. However, at 10^{-4} M haloperidol did not diminish stimulation of striatal Na–K ATPase activity by DA or apomorphine. Comparable studies with haloperidol have not been reported. Desaiah and Ho [18] demonstrated that chlorpromazine, another DA receptor blocking drug, inhibited the DA stimulation of the Na–K ATPase activity of whole mouse brain synaptosomes. However, the fact that chlorpromazine and other tricyclic antipsychotics are potent inhibitors of (basal) Na–K ATPase activity [39] hampers interpretation of this effect. Therefore we did some

experiments with flupenthixol, a tricyclic thioxanthene derivative exhibiting *cis–trans* geometrical isomerism. *cis*-Flupenthixol is a clinically effective, potent DA antagonist, whereas the *trans* isomer is much less active in DA receptor blocking tests [40–42]. We found that both flupenthixol isomers strongly inhibit DA activation of striatal Na–K ATPase activity at concentrations (3.75 to 5×10^{-5} M) which inhibit basal enzyme activity to a limited extent only. Obviously there is no relation between the DA receptor blocking activity of the drugs and their inhibitory effect on DA stimulation of Na–K ATPase; the *trans* isomer is even more potent in diminishing DA stimulation of the enzyme. So in our view the inhibitory effect of chlorpromazine as described by Desaiah and Ho [18] should not be interpreted as an indication for a relation between Na–K ATPase and DA receptor mechanisms. The same applies to the inhibitory effect of fluphenazine on the DA stimulation of striatal Na–K ATPase activity, described in the afore-mentioned publication of Akagawa and Tsukada [17].

Since the DA receptor blockers do not specifically affect the Na–K ATPase stimulation, the apomorphine effect probably is not related with its DA agonistic action. In order to get more information on this question we studied whether other drugs known as DA agonists could stimulate striatal Na–K ATPase activity too. Two of these drugs, bromocriptine, an ergot derivative [43], and piribedil, a non-catechol analogue of DA [44], did not show any effect on the enzyme activity. A third compound, lergotrile, another ergot derivative [45], caused a slight but statistically significant Na–K ATPase activation. Only ADTN, a rigid DA analogue [46], was found to be a potent stimulator of the striatal enzyme, about equally effective as DA. So, three out of four of these agonistic drugs show no or very little effect, which is in line with the suggestion of absence of a relation between DA agonistic action and Na–K ATPase stimulation. However, interpretation of these results is complicated by the fact that the DA agonistic action of these 3 drugs is clearly demonstrated only in *in vivo* experiments, while *in vitro* data are much less convincing and are difficult to interpret [43, 44, 47]. On the other hand, the fact that ADTN stimulates striatal Na–K ATPase activity does not upset our suggestion, as the ADTN effect, just like that of apomorphine, can be explained by the structural resemblance between these drugs and DA, as will be discussed below.

In the literature some authors report inhibition of the stimulatory effect of NA on brain Na–K ATPase activity by adrenergic receptor blockers. We investigated whether these blockers also reduced stimulation of the striatal enzyme by NA. For comparison the effect on cerebral cortex and cerebellum was studied too.

The α adrenoceptor antagonist phentolamine [48] did not affect the NA stimulation of the Na–K ATPase activity of the three brain regions studied, unless the NA stimulated enzyme activities were corrected for the stimulatory effect of phentolamine on the basal activities. As this, however, is a questionable procedure, we conclude that phentolamine has no influence on the NA susceptible Na–K ATPase activities. The absence of an effect of the α blocker on the stimulated Na–K ATPase activity of rat striatal homogenates confirms the results obtained by Akagawa and Tsukada [17]. These authors, however, did not mention whether the blocker influenced basal activity, neither did they study other brain regions. By contrast, three groups of investigators reported inhibition of NA stimulation of brain Na–K ATPase activity by phentolamine in similar concentrations to those we used. Meier-Ruge and Iwangoff [19] did so, using cat and beef cortex homogenates. An effect of phentolamine on basal activity was not mentioned. Gilbert *et al.* [9] reported complete blocking of the NA effect on Na–K ATPase activity of rat cerebral cortex synaptosomes by phentolamine, without any effect on the basal activity. However, their experimental proof is very meagre: the mean of only two observations is presented. Stimulation of the basal enzyme activity as we have seen was also described by Wu and Phillis [20]. But even without correction for this effect, these authors found considerable inhibition by phentolamine of the NA stimulated Na–K ATPase activity of rat cerebral cortex synaptosomes.

We have tried to gather more information on the possible relation between CA susceptible Na–K ATPase and α adrenergic receptor mechanisms by studying the effect of another antagonist, viz. dihydroergotoxine [49], and an agonist, viz. clonidine [50]. As to dihydroergotoxine, we did not find any inhibitory effect on the NA stimulation of the Na–K ATPase activity in any brain region. This result differs from the findings of Meier-Ruge and Iwangoff [19], who observed a strong inhibitory effect of this drug on the stimulated Na–K ATPase activity of cat and beef brain cortex, although in another paper of these authors [51] only a very small effect of dihydroergotoxine was reported. As to the α agonist clonidine, no stimulation of the striatal Na–K ATPase activity was found. Our conclusion is that a relation between CA susceptible Na–K ATPase and α adrenergic receptor mechanisms is unlikely.

Our experiments also indicate absence of a relation with β adrenergic receptor mechanisms, as we did not find an inhibitory effect of the β blocker propranolol [48] on the NA stimulation of Na–K ATPase activity of any brain region. Propranolol does inhibit Na–K ATPase, but basal and NA stimulated activities to about the same extent. Concerning the striatum, our results (again) agree with those of Akagawa and Tsukada [17]. But as to the cortex, our results are (again) at variance with those of Meier-Ruge and Iwangoff [19] and of Wu and Phillis [20], as these groups both found strong inhibition of the NA stimulation by propranolol. Inhibiton of the basal Na–K ATPase activity is reported by various investigators [13, 16, 52].

Summarizing the results of our experiments with DA and NA receptor agonists and antagonists, we have to conclude that they do not yield sufficient evidence for assuming a relation between CA susceptible Na–K ATPase activity and catecholaminergic receptor mechanisms. Our findings naturally do not preclude that stimulation of brain Na–K

ATPase activity by catecholamines has physiological meaning *in vivo*. It is, however, very difficult to get direct experimental evidence for such an *in vivo* role. We have chosen the following indirect approach to this problem: it if could be established that the CA susceptible Na–K ATPase activity is only a fraction of the total Na–K ATPase activity with a specific localization, this would be an indication for a specific role of this enzyme fraction in a certain site. For this purpose we studied the effect of lesions on the DA susceptible Na–K ATPase activity of striatal homogenates. After implantation of 6-OHDA into the nigrostriatal tract, causing a very extensive degeneration of striatal DA nerve terminals, no difference was seen in the DA stimulation of the striatal enzyme between the lesioned and the non-lesioned side of the animals. So we have to conclude that striatal DA susceptible Na–K ATPase is not exclusively or predominantly localized in DA nerve terminals. However, a localization of the DA susceptible enzyme postsynaptically, in structures innervated by DA terminals, could not be excluded yet. To examine this possibility we used kainic acid. Intrastriatal injection of this rigid glutamate analogue causes degeneration of neurons with cell bodies near the injection site (viz. gaba-ergic and cholinergic neurons), while rather specifically sparing axons passing through or terminating in the striatum (e.g. dopaminergic ones) [53]. From the large decrease of striatal DA stimulated adenylate cyclase activity and DA receptor binding after injection of kainic acid [54, 55], one can infer that this drug induces disappearance of a considerable fraction of the neurons innervated by the dopaminergic nigrostriatal neurons. So, if DA susceptible Na–K ATPase is specifically localized postsynaptically with regard to the DA terminals, one should observe a reduction of the DA susceptibility of the enzyme activity after kainic acid. We did find a decrease of the striatal DA stimulated Na–K ATPase activity, but as the lesion affected the basal enzyme activity to the same extent, we have to conclude that the enzyme which disappeared is not distinguishable by a greater susceptibility for DA from the remaining enzyme.

Summarizing, we can say that the results of our lesion experiments suggest that DA susceptible striatal Na–K ATPase activity is neither specifically localized in DA neurons, nor in DA innervated neurons. As it is hard to imagine that a specific DA susceptible enzyme should be localized outside dopaminergic systems, we conclude that DA susceptibility is a general, non-specific characteristic of brain Na–K ATPase activity.

Some authors have suggested that catecholamines stimulate brain Na–K ATPase activity indirectly by abolishing the inhibitory effect of divalent metal ions on the enzyme [8, 15, 21]. Catecholamines are indeed known to be strong chelators of a number of metal ions [56]. This explanation agrees with (and is partly based on) the fact that chelators such as EDTA and EGTA can mimic the effects of catecholamines [8, 15]. We have shown that striatal Na–K ATPase activity also is stimulated by EDTA, and moreover that the effect of a submaximal concentration of EDTA is only partly additive with that of 10^{-4} M DA, never exceeding a certain maximal stimulation. Studies concerning the nature of the metal ion responsible for the CA effect resulted in the suggestion that ferrous ions are involved [15, 21]. Other authors [8, 14] suggested involvement of calcium ions, but their arguments are less convincing. Our results support the idea that such an unspecific mechanism might be responsible for the stimulatory effect of catecholamines on Na–K ATPase: we found no relation between the extent of the effect and the endogenous amine content of brain tissue, no convincing evidence of involvement of CA receptors in the effect and no specific localization of the CA susceptible enzyme.

Studying the effect of dopaminergic drugs, we found a strong stimulatory action by two DA receptor agonists, viz. apomorphine and ADTN. We have not attached much value to this observation, as the stimulatory effect of these drugs probably has to be explained also by chelation of inhibiting metal ions. Both drugs are conformationally restricted analogues of DA, having a catechol group and an amine group. Experiments with different phenylalkyl derivatives have shown that the presence of these groups is a prerequisite for a stimulatory effect on Na–K ATPase activity [5, 15].

The tentative conclusion that the stimulatory effect of catecholamines on Na–K ATPase activity is probably brought about via a non-specific chelation mechanism as outlined above does not necessarily exclude that this phenomenon plays a physiological part *in vivo*. Metal ions are present in brain tissue at concentrations sufficient to inhibit Na–K ATPase activity [56–58]. The rather high CA concentrations required for their *in vitro* effect are no argument against an *in vivo* role, because very little is known about the local *in vivo* CA concentrations and about the minimal change of Na–K ATPase activity which has consequences for the functioning of neurons. Besides, it cannot be excluded that the enzyme has become less sensitive *in vitro* by the homogenization procedure (see ref 16).

Acknowledgements—We wish to express our gratitude to Dr. B. J. van Zwieten-Boot for introducing us into the studies on catecholamine stimulated Na–K ATPase. The technical assistance of Miss M. H. G. Scheffers and Mr. W. F. Maas is much appreciated. We are also indebted to Prof. Dr. E. L. Noach for his critical evaluation of the manuscript. We thank Dr. B. H. C. Westerink and the pharmaceutical companies mentioned in Materials and Methods for their gifts of drugs.

REFERENCES

1. J. C. Skou, *Physiol. Rev.* **5**, 596 (1965).
2. S. E. Vizi, in *Subcortical Mechanisms and Sensorimotor Activities* (Ed. T. L. Frigyesi), p. 63. Huber, Bern (1975).
3. D. M. Paton, in *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines* (Ed. D. M. Paton), p. 155. Raven Press, New York (1976).
4. T. D. White, in *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines* (Ed. D. M. Paton), p. 175. Raven Press, New York (1976).
5. A. Schaefer, G. Unyi and A. K. Pfeifer, *Biochem. Pharmac.* **21**, 2289 (1972).
6. K. Yoshimura, *J. Biochem., Tokyo* **74**, 389 (1973).

7. P. Iwangoff, A. Chappuis and A. Enz, *IRCS Medical Science* 73–8, 3-10-19 (1973).
8. T. Godfraind, M.-C. Koch and N. Verbeke, *Biochem. Pharmac.* **23**, 3505 (1974).
9. J. C. Gilbert, M. G. Wyllie and D. V. Davison, *Nature, Lond.* **255**, 237 (1975).
10. A. H. Demirjian, J. E. Haroutunian and N. H. Yessaian, *Vopr. Biokhim. Mozga* **10**, 137 (1975).
11. D. Desaiah and I. K. Ho, *Eur. J. Pharmac.* **40**, 255 (1976).
12. J. G. Logan and D. J. O'Donovan, *J. Neurochem.* **27**, 185 (1976).
13. P. L. Jeffrey and M. E. Gibbs, *Pharmac. Biochem. Behav.* **5**, 571 (1976).
14. S. L. Lee and J. W. Phillis, *Can. J. Physiol. Pharmac.* **55**, 961 (1977).
15. T. D. Hexum, *Biochem. Pharmac.* **26**, 1221 (1977).
16. P. V. Sulakhe, S.-H. Jan and S. J. Sulakhe, *Gen. Pharmac.* **8**, 37 (1977).
17. K. Akagawa and Y. Tsukada, *J. Neurochem.* **32**, 269 (1979).
18. D. Desaiah and I. K. Ho, *Biochem. Pharmac.* **26**, 2029 (1977).
19. W. Meier-Ruge and P. Iwangoff, *Postgrad. Med. J.* **52** (Suppl. 1) 47 (1976).
20. P. H. Wu and J. W. Phillis, *Gen. Pharmac.* **9**, 421 (1978).
21. A. Schaefer, A. Seregi and M. Komlós, *Biochem. Pharmac.* **23**, 2257 (1974).
22. B. J. Van Zwieten-Boot and A. Petri-Bot, *Eur. J. Pharmac.* **39**, 245 (1976).
23. J. A. Van der Krogt, R. D. M. Belfroid and W. F. Maas, in *Proceedings of the European Society for Neurochemistry* (Ed. V. Neuhoff), Vol. 1, p. 541. Verlag Chemie, Weinheim (1978).
24. J. F. R. König and R. A. Klippel, *The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Williams & Wilkins, Baltimore (1963).
25. P. G. Smelik, *Neuroendocrinology* **2**, 247 (1967).
26. B. H. C. Westerink and J. Korf, *J. Neurochem.* **29**, 697 (1977).
27. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
28. S. L. Bonting, in *Membranes and Ion Transport* (Ed. E. E. Bittar), Vol. 1, p. 257. Wiley-Interscience, London (1970).
29. M. Arluison, Y. Agid and F. Javoy, *Neuroscience* **3**, 657 (1978).
30. T. Grisar, J.-M. Frere and G. Franck, *Brain Res.* **165**, 87 (1979).
31. P. M. Hudgins and G. H. Bond, *Biochem. biophys. Res. Commun.* **77**, 1024 (1977).
32. L. Josephson and L. C. Cantley, *Biochemistry* **16**, 4572 (1977).
33. L. C. Cantley, L. Josephson, R. Warner, M. Yanagisawa, C. Lechene and G. Guidotti, *J. biol. Chem.* **252**, 7421 (1977).
34. E. E. Quist and L. E. Hokin, *Biochem. biophys. Acta* **511**, 202 (1978).
35. P. M. Hudgins and G. H. Bond, *Res. Commun. Chem. Path. Pharmac.* **23**, 313 (1979).
36. G. Di Chiara and G. L. Gessa, *Adv. Pharmac. Chemother.* **15**, 88 (1978).
37. A. Schaefer, A. Seregi and M. Komlós, *J. Pharm. Pharmac.* **29**, 117 (1977).
38. N.-E. Andén, S. G. Butcher, H. Corrodi, K. Fuxe and U. Ungerstedt, *Eur. J. Pharmac.* **11**, 303 (1970).
39. P. Palatini, *Gen. Pharmac.* **9**, 215 (1978).
40. I. Møller-Nielsen, V. Pedersen, M. Nymark, K. F. Frank, V. Boeck, B. Fajlland and A. V. Christensen, *Acta pharmac. tox.* **32**, 353 (1973).
41. R. J. Miller, A. S. Horn and L. L. Iversen, *Molec. Pharmac.* **10**, 759 (1974).
42. D. R. Burt, S. J. Enna, I. Creese and S. H. Snyder, *Proc. natn. Acad. Sci. U.S.A.* **72**, 4655 (1975).
43. K. Fuxe, B. B. Fredholm, S. O. Ögren, L. F. Agnati, T. Hökfelt and J. Å. Gustafsson, *Acta Endocrin.* **88** (Suppl. 216), 27 (1978).
44. S. Consolo, R. Fanelli, S. Garattini, D. Ghezzi, A. Jori, H. Ladinsky, V. Marc and R. Samanin, in *Advances in Neurology* (Eds. D. B. Calne, T. N. Chase and A. Barbeau), Vol. 9, p. 257. Raven Press, New York (1975).
45. J. A. Clemens, C. J. Shaar, E. B. Smalstig, N. J. Bach and E. C. Kornfeld, *Endocrinology* **94**, 1171 (1974).
46. L. L. Iversen, A. S. Horn and R. J. Miller, in *Advances in Neurology* (Eds. D. B. Calne, T. N. Chase and A. Barbeau), Vol. 9, p. 197. Raven Press, New York (1975).
47. J. W. Kebabian and P. R. Kebabian, *Life Sci.* **23**, 2199 (1978).
48. M. Nickerson and B. Collier, in *The Pharmacological Basis of Therapeutics*, 5th Edn (Eds. L. S. Goodman and A. Gilman), p. 533. MacMillan, New York (1975).
49. R. Markstein and H. Wagner, *Gerontology* **24** (Suppl. 1), 94 (1978).
50. L. Finch, R. E. Buckingham, R. A. Moore and T. J. Bucher, *J. Pharm. Pharmac.* **27**, 181 (1975).
51. W. Meier-Ruge, A. Enz, P. Gygax, O. Hunziker, P. Iwangoff and K. Reichlmeier, in *Aging* (Eds. S. Gershon and A. Raskin), Vol. 2, p. 55. Raven Press, New York (1975).
52. P. Iwangoff, A. Enz en A. Chappuis, *Experientia* **30**, 688 (1974).
53. E. G. McGeer, J. W. Olney and P. L. McGeer (Eds.), *Kainic Acid as a Tool in Neurobiology*. Raven Press, New York (1978).
54. E. G. McGeer, V. T. Innanen and P. L. McGeer, *Brain Res.* **118**, 356 (1976).
55. S. Govoni, V. R. Olgiati, M. Trabucchi, L. Garau, E. Stefanini and P. F. Spano, *Neurosci. Lett.* **8**, 207 (1978).
56. R. W. Colburn and J. W. Maas, *Nature, Lond.* **208**, 37 (1965).
57. T. D. Hexum, *Biochem. Pharmac.* **23**, 3441 (1974).
58. K. S. Rajan, R. W. Colburn and J. M. Davis, *Life Sci.* **18**, 423 (1976).