Biochemical Pharmacology, Vol. 31, No. 13, pp. 2231–2236, 1982.
Printed in Great Britain.

0006-2952/82/132231-06 $03.00/0

RECEPTOR INDEPENDENT STIMULATORY EFFECT OF NORADRENALINE ON Na,K-ATPase IN RAT BRAIN HOMOGENATE

ROLE OF LIPID PEROXIDATION

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(*Received* 4 *June* 1981; *accepted* 18 *January* 1982)

Abstract—The effect of different adrenoceptor agonists on Na,K-ATPase activity and lipid peroxidation of rat brain homogenate was studied. Drugs which enhanced Na,K-ATPase activity—noradrenaline, adrenaline and oxymethazoline—were found to inhibit endogenous membrane lipid peroxidation. Other drugs—phenylephrine, xylazine and clonidine—which did not cause any change in the enzyme activity did not influence lipid peroxidation either. No increase of Na,K-ATPase activity by noradrenaline could be detected after preincubation of the homogenate for 5 min at 37°. During this time endogenous lipid peroxidation of considerable extent could be observed. It is concluded that there is no correlation between the adrenoceptor agonist feature of noradrenaline and its stimulatory effect on Na,K-ATPase activity of rat brain homogenate. However, it seems likely that in rat brain homogenate the increase of Na,K-ATPase activity and inhibition of endogenous lipid peroxidation by noradrenaline are related.

It has been suggested that synaptic membrane Na,K-ATPase may play a role in the regulation of transmitter release. Experimental conditions, known to inhibit [1, 2, 4] or stimulate [3, 4] Na,K-ATPase activity in some tissues, were shown to elicit [1, 2, 4] or inhibit [3, 4] transmitter release from nerve terminals, respectively. Presynaptic α-adrenoceptor mediated [5] negative feedback regulation of noradrenergic neurotransmission [6], as well as inhibition of acetylcholine release by noradrenaline [2], were suggested to be possible results of stimulation of Na,K-ATPase by noradrenaline [3, 4]. Noradrenaline induced hyperpolarization of central neurones was also regarded as a consequence of noradrenaline-stimulation of neuronal Na,K-ATPase [7, 8].

Since 1972, when Schaefer *et al.* [9] found that catecholamines increased Na,K-ATPase activity in the particulate fractions of rat brain by relieving its inhibition by a soluble factor, a series of papers dealing with the effect of biogenic amines has appeared. Increase of Na,K-ATPase activity in the presence of catecholamines has been reported for microsomal [10–12], synaptosomal [6, 13, 14] and synaptic membrane fractions [15–19]. However, recent results have clearly shown that increase of Na,K-ATPase activity in these preparations is due to the reversal by catecholamines of a metal ion [12, 17], vanadate [16, 20] or soluble factor induced inhibition [9, 10, 21–24]. When microsomes [12, 21] or osmotically disrupted synaptosomes [17, 18, 20] have been studied, using ATP free of metal contamination, no activation by noradrenaline could be observed in these subfractions. The only preparation where catecholamines can consistently enhance Na,K-ATPase activity, even with metal free ATP, is the homogenate of total brain [25–28] or of different brain areas [29–34]. However, the interpretations of the results obtained with homogenate are contradictory. Some authors have suspected an unspecific mechanism for the effect of catecholamines [27, 28, 31, 34]. Others have suggested that specific adrenoceptors are involved in the catecholamine induced increase of brain Na,K-ATPase [26, 30, 32, 33]. In view of the observation [11] that ascorbic acid induced lipid peroxidation of membranes results in inhibition of Na,K-ATPase, we have examined the effect of different agonists and antagonists and of other conditions on the extent of lipid peroxidation and Na,K-ATPase activity of homogenate in order to determine if any relationship exists between the two which might explain observations described above.

MATERIALS AND METHODS

Rats weighing 150–200 g were decapitated. Cerebral cortex (2 g) was homogenized in 20 ml 0.3 M sucrose by using a Potter–Elvehjem homogenizer and centrifuged at 1000 *g* for 10 min. The supernatant was diluted with distilled water to get protein concentration of 1 mg/ml. The procedure was performed at 4°.

Measurement of Na,K-*ATPase activity*

For total ATPase assay the medium was of the following composition: Tris–HCl buffer pH 7.4 50 mM; $MgCl_2$ 5 mM; NaCl 100 mM; KCl 20 mM; ATP 5 mM. For Mg-ATPase assay the medium contained: Tris buffer pH 7.4 50 mM; $MgCl_2$ 5 mM;

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ATP 5 mM. The final volume was 2 ml. After preincubation for 1 min, the reaction was started by addition of the enzyme (0.15–0.2 mg protein). The incubation was carried out at 37° for 10 min and was stopped by addition of 1 ml trichloroacetic acid (TCA, 20%). The phosphate content was determined by the method of Fiske and SubbaRow [35].

In the experiments shown in Table 3, the ATPase assay was performed in the same medium and volume but the reaction was started after 5 min preincubation of homogenate by ATP (see Results).

The Na,K-ATPase activity was calculated by subtracting Mg-ATPase activity from the total ATPase activity and expressed in μmole P_i/mg protein/hr/(μmole Pi/mg/hr).

Protein content of the homogenate was measured as described by Lowry *et al.* [36].

Lipid peroxide measurement

Lipid peroxidation was measured by thiobarbituric acid reaction as described by Wilbur *et al.* [37]. The amount of malondialdehyde (MDA) formed was calculated by the molar extinction coefficient $\varepsilon_{530} = 1.56 \times 10^5$ cm²/mmole, reported by Sinnhuber *et al.* [38] and was used to express the extent of peroxidation.

Drugs

These include noradrenaline HCl (BDH Chemicals Ltd., Poole, U.K.), adrenaline HCl (Koch Light Lab. Ltd., Colnbrook, U.K.), oxymethazoline (Merck, Darmstadt, West Germany), phenylephrine HCl (ROTA), clonidine HCl (Boehringer Co. Ltd., London, U.K.), xylazine (Bayern), phenoxybenzamine (CIBA), yohimbine HCl (Sigma Chemical Co., St. Louis, MO), phentolamine methanesulphonate (CIBA), prazocine HCl (Pfizer, Sandwich, U.K.), propranolol (ICI), ATP disodium salt, vanadate free (Sigma, Lot. No 96C-7170). Other chemicals were purchased from Reanal (Budapest).

RESULTS

Effect of different adrenoceptor agonists and antagonists on Na,K-*ATPase of rat brain homogenate*

Figure 1 shows the effect of six different adrenoceptor agonists. Noradrenaline, adrenaline known as α_1 and α_2 receptor agonists increased the enzyme activity. Oxymethazoline, an α_2 receptor agonist,

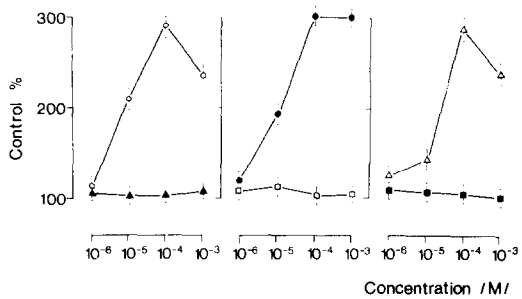

Fig. 1. Effect of different adrenoceptor agonists on Na,K-ATPase of rat brain homogenate. Na,K-ATPase activity of the homogenates: 6.6 ± 1.2 μmole Pi/mg/hr was taken as 100%. ○——○, noreadrenaline; ▲——▲, phenylephrine; ●——●, adrenaline; □——□, clonidine; △——△, oxymethazoline; ■——■, xylazine. Each point represents an average of three experiments in duplicate ± S.E.M.

was also capable of activating the enzyme to the same extent as noradrenaline and adrenaline. All of the three drugs were most effective at 10^{-4} M concentration. However, other α_2 receptor agonists, clonidine and xylazine were completely ineffective over the concentration range 10^{-6} to 10^{-3} M. Phenylephrine, effective only on α_1 receptor, did not change Na,K-ATPase activity either (Fig. 1). Isoprenaline, a β receptor agonist, increased the enzyme activity similarly to noradrenaline, adrenaline and oxymethazoline (data not shown).

Phentolamine, an antagonist which can act on both α_1 and α_2 receptors, slightly stimulated basal Na,K-ATPase activity. Similar effects could be produced by prazocine (Table 1). Yohimbine (10^{-4} M), a specific α_2 antagonist, had a most pronounced effect on the enzyme: it increased basal activity from 7.6 ± 0.9 to 11.5 ± 1.2 μmole Pi/mg/hr (Fig. 2b).

Taking into account the absolute values, there was no decrease in the effect of noradrenaline (10^{-4} M) in the presence of these antagonists (Fig. 2 and Table 1). The antagonist, propranolol, did not change the stimulatory effect of noradrenaline either (Table 1). Phenoxybenzamine (10^{-5} and 10^{-4} M) in our preparation caused no significant change in the basal activity of the enzyme (Fig. 2a). However, it was the only drug among the antagonists which was able to decrease the enzyme stimulation by noradrenaline (Fig. 2a). The question arises now, whether or not this effect of phenoxybenzamine is a receptor

Table 1. Effect of phentolamine, prazocine and propranolol on Na,K-ATPase activity and its stimulation by noradrenaline

		Na,K-ATPase activity μmole Pi/mg/hr	
		−NA	+NA (10^{-4} M)
—		4.99 ± 0.7	14.73 ± 1.3
Phentolamine	10^{-5} M	5.68 ± 0.1	14.8 ± 0.4
	10^{-4} M	6.65 ± 0.25	16.2 ± 0.95
Prazocine	10^{-5} M	5.7 ± 0.06	13.3 ± 0.4
	10^{-4} M	6.2 ± 0.4	15.8 ± 0.33
Propranolol	10^{-5} M	5.4 ± 1.4	15.8 ± 2.4
	10^{-4} M	5.7 ± 0.9	17.1 ± 2.6

Each point represents an average of three duplicate experiments ± S.E.M.
NA; noradrenaline.

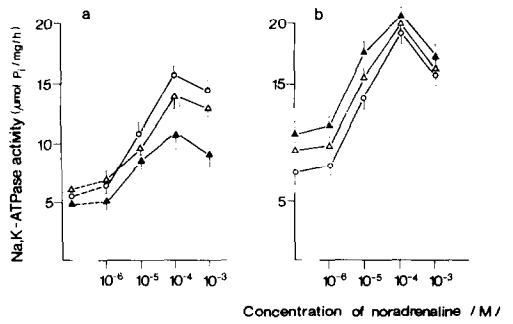

Fig. 2. Effect of phenoxybenzamine (a) and yohimbine (b) on the stimulation of Na,K-ATPase by noradrenaline. (a) ○——○, noradrenaline alone; △——△, noradrenaline with 10^{-5} M phenoxybenzamine; ▲——▲, noradrenaline with 10^{-4} M phenoxybenzamine. (b) ○——○, noradrenaline alone; △——△, noradrenaline with 10^{-5} M yohimbine; ▲——▲, noradrenaline with 10^{-4} M yohimbine. Each point represents an average of three duplicate experiments ± S.E.M.

Table 2. Effect of different adrenoceptor agonists and antagonists on endogenous lipid peroxidation of homogenate

	MDA formation (%)
Control	100
Noradrenaline	0.8 ± 0.8
Adrenaline	1.2 ± 0.7
Oxymethazoline	0.6 ± 0.6
Phenylephrine	102.0 ± 3.1
Xylazine	99.6 ± 1.56
Clonidine	99.9 ± 1.0
Yohimbine	55.5 ± 1.6
Phentolamine	86.2 ± 1.3

MDA formed in control experiments (100%): 3.1 ± 0.07 nmole/mg/10 min.

All drugs were tested in 10^{-4} M concentration. Protein concentration in the medium was 230–250 μg/ml. Each point represents an average of three experiments ± S.E.M.

mediated process. Therefore, its effect on the enzyme activation enhanced by either EGTA [27, 28, 31] or Co^{2+} [29] was also studied. These conditions are known to be unspecific, independent of receptor mediation. The effect of phenoxybenzamine under these circumstances are shown in Fig. 3. The stimulatory effect of EGTA and of Co^{2+} (10^{-4} M) could also be decreased by phenoxybenzamine to a similar extent than that of noradrenaline.

Effect of different adrenoceptor agonists and antagonist on the endogenous lipid peroxidation of homogenate

Table 2 shows how endogenous lipid peroxidation of homogenate was changed in the presence of different agonists and antagonists measuring under the same conditions as the assay of Na,K-ATPase was carried out. Noradrenaline, adrenaline and oxymethazoline (10^{-4} M) completely prevented MDA formation in the medium. Phenylephrine, xylazine and clonidine failed to influence lipid peroxide formation under these conditions. Yohimbine (10^{-4} M) had a distinct effect on lipid peroxidation. Phentolamine decreased lipid peroxidation of homogenate, however, to a lesser extent than yohimbine. Phenoxybenzamine (10^{-4} M) did not cause any change in the extent of lipid peroxidation either in the absence or in the presence of noradrenaline (not shown).

Table 3. Correlation between the activity of Na,K-ATPase and lipid peroxidation in homogenate under different incubation conditions with noradrenaline

	Activity of Na,K-ATPase μmole/Pi/mg/hr	Lipid peroxide formation nmole/MDA/mg
* Incubation without ATP (5 min)	—	0.93 ± 0.03
† Preincubation (5 min) Incubation (10 min)	4.34 ± 0.8	3.41 ± 0.09
‡ Preincubation with 10^{-4} M noradrenaline (5 min) Incubation (10 min)	11.31 ± 1.2	0.04 ± 0.04
§ Preincubation (5 min) Incubation with 10^{-4} M noradrenaline (10 min)	5.4 ± 0.87	0.86 ± 0.02

In these experiments ATPase reaction was started by ATP after preincubation of homogenate for 5 min.

* After 5 min incubation without ATP homogenate was precipitated by TCA and lipid peroxidation was measured.

† After 5 min preincubation of homogenate reaction was started by ATP and incubated for further 10 min.

‡ The same as † but noradrenaline (10^{-4} M) was present during the preincubation period.

§ The same as † but noradrenaline was added together with ATP after 5 min preincubation of homogenate.

Each point represents an average of three duplicate experiments ± S.E.M.

Lack of the stimulatory effect of noradrenaline on Na,K-*ATPase in homogenate preincubated for 5 min; relation to lipid peroxidation*

In these experiments homogenate was preincubated for 5 min at 37° in the reaction medium with or without noradrenaline. The reaction was started by ATP after this preincubation period and the incubation was followed for further 10 min period. Table 3 shows that, if homogenate had been preincubated with noradrenaline for 5 min, the enzyme activity was increased from 4.34 ± 0.8 to 11.31 ± 1.2 μmole Pi/mg/hr. In contrast, if homogenate had been preincubated for 5 min and noradrenaline was added together with ATP at 5 min, only a slight increase could be obtained (from 4.34 ± 0.8 to 5.4 ± 0.87 μmole Pi/mg/hr). Under these conditions when measuring the lipid peroxidation of homogenate, we found that noradrenaline when it was present in the preincubation period, i.e. when Na,K-ATPase activity was increased, completely abolished lipid peroxide formation (from 3.41 ± 0.09 to 0.04 ± 0.04 nmole MDA/mg). When noradrenaline was added after 5 min preincubation, the amount of lipid peroxides (0.86 ± 0.02 nmole MDA/mg) measured after a further 10 min period was almost identical to that measured after 5 min incubation without noradrenaline (0.93 ± 0.03 nmole MDA/mg). This finding indicates that addition of noradrenaline stopped further lipid peroxide formation.

DISCUSSION

It has been well known for several years that catecholamines can increase Na,K-ATPase activity in a brain homogenate [25–34]. However, little information is available on the mechanism of this action. It has been proposed that specific receptors are involved in the effect of catecholamines on Na,K-ATPase of homogenate [26, 30, 32, 33]. This suggestion was based on experiments where some adrenoceptor agonists were found to be effective on Na,K-ATPase of the homogenate and some antagonist decreased the effect of these agonists.

In contrast, Van der Krogt and Belfroid [34], who examined in detail the effect of several agonist and antagonist on the activity of the enzyme, have come to the conclusion that in the homogenate the effect of catecholamines is most probably not related to specific adrenoceptors. In the present work, we have also examined the effect of different adrenoceptor agonists and found that some of them (adrenaline, noradrenaline, isoproterenol, oxymethazoline) can increase Na,K-ATPase activity, while others (phenylephrine, xylazine, clonidine) were ineffective (Fig. 1). In addition, none of the receptor antagonists studied (phentolamine, yohimbine, prazocine, phenoxybenzamine) could decrease the effect of noradrenaline except phenoxybenzamine (Fig. 2., Table 1). However, phenoxybenzamine is known to have a non-specific inhibitory effect on Na,K-ATPase activity [39] provided that it is not already inhibited by soluble fraction [22] or by metal ions [17]. By our finding that phenoxybenzamine could antagonize the enhancement of Na,K-ATPase activity caused by

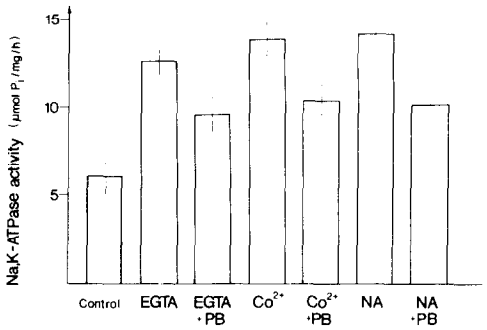

Fig. 3. Effect of phenoxybenzamine (PB, 10^{-4} M) on the stimulation of Na,K-ATPase by EGTA (10^{-4} M), $CoCl_2$ (10^{-4} M) and noradrenaline (NA) (10^{-4} M). Each point represents an average of two duplicate experiments ± S.E.M.

EGTA or Co^{2+} (Fig. 3) the nonspecific nature of its effect is supported.

On the basis of these results, no correlation can be seen between the effect of the drugs on Na,K-ATPase and their adrenoceptor agonist or antagonist feature. However, due to the heterogeneity of the preparation, no firm conclusion can be drawn: the homogenate contains Na,K-ATPase originating from different sites of the cell, each of which may respond differently to an agonist and/or antagonist. Na,K-ATPase activity measured in homogenates in our and other's experiments must be a net effect. Therefore, in order to explain why Na,K-ATPase activity is increased in the presence of noradrenaline and some other receptor agonists, it seems advisable not to give more attention to the receptors but to find common characteristics or effects of these drugs which may be related to their effect on the enzyme.

Schaefer *et al.* [9] have shown that catecholamines increase Na,K-ATPase activity in various particulate fractions of rat brain by antagonizing the effect of an endogenous inhibitor present in the cytosol. One soluble inhibitor has been identified as ascorbic acid [10] and its lipid-peroxidation inducing effect has been shown to be responsible for the inhibition of the enzyme [11]. Recently similar observation has been published by Matsuda *et al.* [40] and Svoboda and Mosinger [21]. Inhibition of Na,K-ATPase by hyperbaric oxygen as a result of lipid peroxidation is demonstrated by Kovachich [41].

The concentration of ascorbic acid in the brain tissue is about 2×10^{-3} M [10, 42, 43]. On the basis of this data, we calculated that in our incubation medium the concentration of ascorbic acid originated from homogenate was about 2×10^{-6} M. This concentration has been shown to be able to inhibit Na,K-ATPase of microsomes [10, 21] by promoting lipid peroxidation [11, 21, 43]. It could be expected that this endogenous ascorbic acid concentration could cause lipid peroxidation in the homogenate also. As shown in Table 2 and Table 3 we could detect time-dependent lipid peroxidation in the homogenate. All those agonists (and antagonists) which were able to enhance enzyme activity, proved to be inhibitors of endogenous lipid peroxidation. Table 2 shows that, in addition to noradrenaline and

adrenaline which have been already shown to inhibit lipid peroxidation of microsomes caused by exogenous ascorbic acid [11] or by cytosol [22], oxymethazoline could completely depress endogenous lipid peroxidation. Drugs ineffective in increasing Na,K-ATPase activity did not influence lipid peroxidation either. These findings strongly suggest that the inhibition of lipid peroxidation is important in the effect of noradrenaline and of other drugs rather than the extent and nature of adrenoceptor activation.

The finding that, in our experiments, yohimbine increased basal activity can also be explained by its pronounced inhibitory effect on lipid peroxidation. Similar explanation might be given for the slight increase in Na,K-ATPase activity by phentolamine in our and other experiments [33, 34]. The same phenomenon might explain the stimulatory effect of dopamine [31, 34], serotonin [30], apomorphine [34] EDTA [29, 34], EGTA [27, 28, 31], Co^{2+} [29] and Mn^{2+} [29] on Na,K-ATPase of homogenate: these substances are potent inhibitors of ascorbic acid induced lipid peroxidation [11].

Further evidence for the role of lipid peroxidation is furnished by the results shown in Table 3. Noradrenaline, when it was preincubated with homogenate, completely prevented lipid peroxidation (from 3.41 ± 0.09 to 0.04 ± 0.04 nmole MDA/mg) and resulted in an increase in Na,K-ATPase activity (from 4.34 ± 0.8 to 11.31 ± 1.2 μmole Pi/mg/hr). However, when homogenate was preincubated for 5 min and noradrenaline was added together with ATP lipid peroxide formation (0.86 ± 0.02 nmole MDA/mg) was consistent to that observed after 5 min incubation of homogenate without noradrenaline and ATP (0.093 ± 0.03 nmole MDA/mg). This fact indicates that adding noradrenaline at 5 min stopped further lipid peroxidation. However, it could not reverse the inhibition of the enzyme caused by lipid peroxidation proceeded in the first 5 min. The activities of Na,K-ATPase in this case (5.4 ± 0.87 μmole Pi/mg/hr) and in that (4.34 ± 0.8 μmole Pi/mg/hr) where lipid peroxidation was not inhibited are very similar, indicating that lipid peroxides formed during 5 min preincubation are enough to cause irreversible inhibiton of Na,K-ATPase. These results may explain the finding of Wu and Phillis that the presence of noradrenaline during the preincubation period was necessary to obtain an increase in Na,K-ATPase of homogenate [33].

We think that the effect of noradrenaline on Na,K-ATPase activity of homogenate is only an apparent stimulation: in reality the increase in the enzyme activity is a consequence of prevention by noradrenaline of lipid peroxidation, which is caused by ascorbic acid present in the homogenate. This inhibitory effect of noradrenaline is most probably due to its ability to chelate metal tracers [10, 12, 21, 24, 34] which are necessary for ascorbic acid induced lipid peroxidation.

Taking into account our present results and recent evidences obtained by others we do not know any preparation or circumstance where direct stimulation of Na,K-ATPase by noradrenaline would be proved. The so called activating action of noradrenaline can be much rather regarded as a protective effect on Na,K-ATPase supressed by metal ion [12, 17], vanadate [18, 44], lipid peroxidation [11, 22–40] or by the simultaneous effect of these inhibitory factors. Thus, the biochemical data available at present concerning the effect of noradrenaline on Na,K-ATPase activity can not support the suggestion that the inhibition of transmitter release might be due to the stimulation of Na,K-ATPase by noradrenaline [45, 46]. However, the possibility cannot be ruled out that a receptor mediated effect of noradrenaline on the enzyme related to transmitter release or membrane polarization is masked by its aspecific action on the heterogenous enzyme preparation. The elucidation of this point, however, requires further study.

Acknowledgement—We are indebted to Mrs. Adél Tarcsafalvi for her valuable technical assitance.

REFERENCES

1. W. D. M. Paton, E. S. Vizi and M. A. Zar, *J. Physiol., Lond.* **215**, 819 (1971).
2. E. S. Vizi, *J. Physiol., Lond.* **226**, 95 (1972).
3. E. S. Vizi, *J. Physiol., Lond.* **267**, 261 (1977).
4. E. S. Vizi, *Neuroscience* **3**, 367 (1978).
5. S. Z. Langer, *Br. J. Pharmac.* **60**, 481 (1977).
6. J. C. Gilbert, M. C. Wyllie and D. V. Davison, *Nature, Lond.* **255**, 237 (1975).
7. B. S. R. Sastry and J. W. Phillis, *Can. J. Physiol. Pharmac.* **55**, 170 (1977).
8. J. W. Phillis, B. S. R. Sastry and P. H. Wu, in *Recent Advances in the Pharmacology of Adrenoceptors* (Eds. E. Szabadi, C. M. Bradshaw and P. Bewan), p. 121. Elsevier/North Holland Biomedical Press, Amsterdam (1978).
9. A. Schaefer, G. Unyi and A. K. Pfeifer, *Biochem. Pharmac.* **21**, 2289 (1972).
10. A. Schaefer, A. Seregi and M. Komlós, *Biochem. Pharmac.* **23**, 2257 (1974).
11. A. Schaefer, M. Komlós and A. Seregi, *Biochem. Pharmac.* **24**, 1781 (1975).
12. T. D. Hexum, *Biochem. Pharmac.* **26**, 1221 (1977).
13. D. Desaiah and I. K. Ho, *Eur. J. Pharmac.* **40**, 255 (1976).
14. D. Desaiah and I. K. Ho, *Biochem. Pharmac.* **26**, 2029 (1977).
15. J. G. Logan and D. J. O'Donovan, *J. Neurochem.* **27**, 185 (1976).
16. P. H. Wu and J. W. Phillis, *Gen. Pharmac.* **9**, 421 (1978)
17. A. Schaefer, M. Komlós and A. Seregi, *Biochem. Pharmac.* **28**, 2307 (1979).
18. V. Ádám-Vizi, M. Ördögh, I. Horváth, J. Somogyi and E. S. Vizi, *J. Neural Transm.* **47**, 53 (1980).
19. L. G. Logan and D. J. O'Donovan, *Biochem. Pharmac.* **29**, 2105 (1980).
20. V. Ádám-Vizi, G. Váradi and P. Simon, *J. Neurochem.* **36**, 1616 (1981).
21. P. Svoboda and B. Mosinger, *Biochem. Pharmac.* **30**, 427 (1981).
22. P. Svoboda and B. Mosinger, *Biochem. Pharmac.* **30**, 433 (1981).
23. K. Goto and R. Tanaka, *Brain Res.* **207**, 239 (1981).
24. A. H. Sawas and J. C. Gilbert, *Biochem. Pharmac.* **30**, 1799 (1981).
25. K. Yoshimura, *J. Biochem. Tokyo* **74**, 389 (1973).
26. P. Iwangoff, A. Enz and A. Chappuis, *Experientia* **30**, 688 (1974).

27. T. Godfraind, M. C. Koch and N. Verbeke, *Biochem. Pharmac.* **23**, 3505 (1974).
28. S. L. Aksentsev, S. V. Konve, T. I. Lyskova, I. M. Okun, R. D. Adzerikho, A. A. Rakovich and A. A. Milyutin, *Biokhimiya U.S.S.R.* **43**, 1492 (1978).
29. P. H. Wu and J. W. Phillis, *Int. J. Biochem.* **10**, 629 (1979).
30. P. H. Wu and J. W. Phillis, *J. Pharm. Pharmac.* **31**, 780 (1979).
31. K. Akagawa and Y. Tsukada, *J. Neurochem.* **32**, 269 (1979).
32. P. H. Wu and J. W. Phillis, *Gen. Pharmac.* **10**, 189 (1979).
33. P. H. Wu and J. W. Phillis, *Int. J. Biochem.* **12**, 353 (1980).
34. J. A. Van der Krogt and R. D. M. Belfroid, *Biochem. Pharmac.* **29**, 857 (1980).
35. C. H. Fiske and Y. SubbaRow, *J. biol. Chem.* **66**, 375 (1925).
36. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
37. K. M. Wilbur, F. Bernheim and O. W. Shapiro, *Archs Biochem. Biophys.* **24**, 305 (1949).
38. R. O. Sinnhuber, T. C. Yu and T. C. Yu, *Fd Res.* **23**, 626 (1958).
39. T. D. Hexum, *Biochem. Pharmac.* **27**, 2109 (1978).
40. T. Matsuda, S. Maeda, A. Baba and H. Iwata, *J. Neurochem.* **32**, 443 (1979).
41. G. B. Kovachich and O. P. Mishra, *J. Neurochem.* **36**, 333 (1981).
42. R. Rajalakshmi and A. J. Patel, *J. Neurochem.* **15**, 195 (1968).
43. A. Seregi, A. Schaefer and M. Komlós, *Experientia* **34**, 1056 (1978).
44. V. Ádám-Vizi, in *Modulation of Neurochemical Transmission* (Ed. E. S. Vizi), p. 259. Pergamon Press, Akadémiai Kiadó (1980).
45. E. S. Vizi, *Progr. Neurobiol.* **12**, 181 (1979).
46. D. A. Powis, *Biochem. Pharmac.* **30**, 2389 (1981).