Drug Development Research 14:285–291 (1988)

Vinpocetine Facilitates Noradrenaline Release in Rat Brain Cortex Slices

E. Schlicker, R. Betz, and M. Göthert

Institut für Pharmakologie und Toxikologie der Rheinischen Friedrich-Wilhelms-Universität Bonn, Bonn 1, Federal Republic of Germany

ABSTRACT

Schlicker, E., R. Betz and M. Göthert: Vinpocetine facilitates noradrenaline release in rat brain cortex slices. Drug Dev. Res. **14**:285–291, 1988.

Rat brain cortex slices preincubated with ^{3}H-noradrenaline were superfused with physiological salt solution, and the effect of vinpocetine on the electrically (3 Hz) evoked ^{3}H overflow was studied. Vinpocetine at 10–100 μmol/liter facilitated the evoked overflow; at 32 and 100 μmol/liter, basal efflux was slightly increased. The facilitatory effect of vinpocetine on the evoked overflow was not altered by desipramine, rolipram (an inhibitor of cAMP phosphodiesterase), or ICS 205-930 ([3α-tropanyl]-1H-indole-3-carboxylic acid ester; an antagonist at 5-HT_3 (M) receptors); it was strongly attenuated by phentolamine. In turn, vinpocetine moderately decreased the facilitatory effect of phentolamine on the evoked overflow. The concentration-response curve of noradrenaline (obtained in the presence of desipramine) for its inhibitory effect on the evoked overflow was not shifted to the right by vinpocetine at 32 and 100 μmol/liter. The present results show that vinpocetine facilitates noradrenaline release. This effect may be related to the blockade of presynaptic α_2-adrenoceptors (although evidence is not unequivocal), but does not appear to involve inhibition of noradrenaline uptake or cAMP phosphodiesterase nor activation of 5-HT_3 (M) receptors. The facilitatory effect of vinpocetine on noradrenaline release occurs at concentrations higher than those obtained under treatment with this drug.

Key words: presynaptic α_2-adrenoceptors, superfusion experiments, aging

INTRODUCTION

Vinpocetine (3α, 16α eburnamenine-14-carboxylic acid ethyl ester) is used for the treatment of mental and neurological symptoms of cerebral dysfunction and for the treatment

Received final version July 27, 1988; accepted August 1, 1988.

Address reprint requests to Dr. E. Schlicker, Institut für Pharmakologie und Toxikologie der Rheinischen Friedrich-Wilhelms-Universität Bonn, Reuterstrasse 2B, D-5300 Bonn 1, FRG.

of ischemic cerebral impairment. The drug was shown to increase noradrenaline levels in the rat brain six hr after intraperitoneal administration [Rosdy et al., 1976]. In a recent study, Pauló et al. [1986] demonstrated that vinpocetine facilitated the electrically evoked ^{3}H-noradrenaline release from strips of rabbit pulmonary artery.

In light of these findings, the aim of the present study was to examine whether vinpocetine also facilitates the electrically evoked ^{3}H-noradrenaline release from rat brain cortex slices. Since this was actually found, the effect of the drug was compared in slices obtained from young adult and aged rats. In addition, experiments were carried out to study the mechanism underlying the facilitatory effect of the drug on noradrenaline release.

MATERIALS AND METHODS

Experiments were performed as described by Schlicker et al. [1988a] with slight modifications. Briefly, slices from the occipitoparietal cortex of young adult (unless stated otherwise) male Wistar rats were preincubated with ^{3}H-noradrenaline 0.1 μmol/liter (60 min, 37°C) and subsequently superfused with physiological salt solution (110 min; 37° C; flow rate, 1 ml/min; for composition of the physiological salt solution, see Schlicker et al. [1988a]. Forty and 90 minutes after onset of superfusion, two 2-min periods of electrical field stimulation were administered to each slice (rectangular pulses of 24 mA and 2 ms; 3 Hz). The radioactivity of the slices and superfusate samples was determined by liquid scintillation counting.

Calculations and Statistics

Tritium efflux into the superfusate was calculated as the fraction of ^{3}H present in the slices at the onset of the respective collection period (fractional rate of ^{3}H efflux). Tritium overflow evoked by the first or second period of electrical field stimulation (S_1 and S_2, respectively) was determined as the ^{3}H overflow in excess of the basal ^{3}H efflux (estimated as described by Schlicker et al. [1988a]) during stimulation and the subsequent 13 min and was expressed as percent of tissue tritium at the onset of the respective stimulation period. To quantify effects of drugs on the electrically evoked and basal ^{3}H efflux, the ratios S_2/S_1 and t_2/t_1 were determined, respectively (t_2 and t_1: fractional rates of ^{3}H efflux in the 5-min samples from the 85th–90th and from the 55th–60th min of superfusion, respectively).

The apparent pA_2 value of phentolamine against noradrenaline was calculated according to Furchgott [1972].

Means ± S.E.M. of n experiments are given throughout the paper. For comparison of two or more than two mean values, Student's t test and Dunnett's test were used, respectively.

Drugs Used

(−)-[ring 2,5,6-^{3}H] noradrenaline (NEN, Dreieich, FRG); desipramine HCl, phentolamine methane sulphonate (CIBA-Geigy, Wehr, FRG); ICS 205-930 (3α-tropanyl)-1H-indole-3-carboxylic acid ester; Sandoz, Basel, Switzerland); (−)-noradrenaline (base; Hoechst, Frankfurt, FRG); rolipram (Schering, Berlin); vinpocetine (Thiemann, Waltrop, FRG) were used.

RESULTS

Effect of Vinpocetine on Tritium Overflow

The basal ^{3}H efflux, expressed as the ratio t_2/t_1 (see Materials and Methods), was 0.79 ± 0.03 in 16 control experiments. Vinpocetine, which was present in the physiological salt solution before and during S_2, did not affect the basal efflux at 1 and 10 μmol/liter, but at 32 and 100 μmol/liter increased it by 20% and 32%, respectively (results not shown).

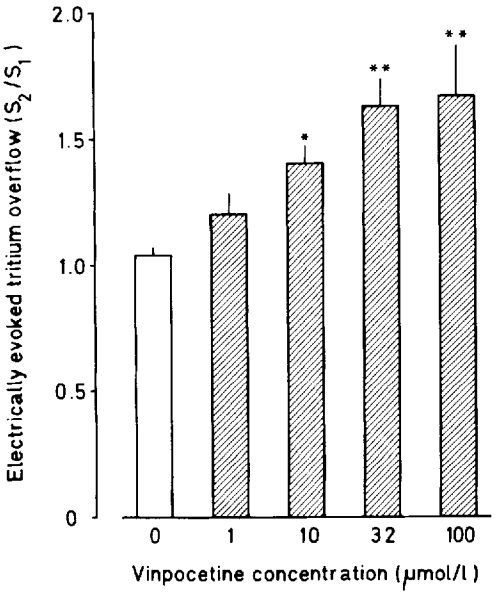

Fig. 1. Effect of vinpocetine on the electrically evoked ^3H overflow from superfused rat brain cortex slices preincubated with ^3H-noradrenaline. Vinpocetine was added to the superfusion medium from the 62nd min of superfusion onward. Tritium overflow was evoked twice, after 40 and 90 min (S_1 and S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1; ^3H overflow evoked by S_2 was 3.84 ± 0.46 nCi in 16 control experiments, corresponding to 4.71 ± 0.75% of tissue tritium). Means ± S.E.M. of 5–18 experiments. *$P < 0.05$; **$P < 0.01$.

The electrically evoked ^3H overflow, expressed as S_2/S_1 (see Materials and Methods; for absolute values, see Fig. 1), was increased by vinpocetine in a concentration-dependent manner; the facilitatory effect of the drug was significant at 10–100 μmol/liter; the degree of facilitation observed at 100 μmol/liter (higher concentrations could not be dissolved in physiological solt solution) was 60% (Fig. 1). The concentration producing 50% of the effect obtained at 100 μmol/liter amounted to 5.6 μmol/liter.

In a separate set of experiments, the effect of vinpocetine at 1, 10, and 32 μmol/liter on the electrically evoked ^3H overflow was studied in cortex slices of 10–14-week, 1-year and 2-year-old rats. The effect of vinpocetine did not exhibit differences between the variously aged animals (n = 5–8; results not shown).

Interaction Experiments

In the first series of experiments (Fig. 2), the interaction of vinpocetine (present in the superfusion medium before and during S_2) with phentolamine, desipramine, ICS 205-930, or rolipram (each drug present throughout superfusion) was studied. The facilitatory effect of vinpocetine on the electrically evoked ^3H overflow was not affected by desipramine (an inhibitor of noradrenaline uptake), ICS 205-930 (a 5-HT_3 (M) receptor antagonist), and rolipram (a cAMP phosphodiesterase inhibitor). In the presence of the α-adrenoceptor antagonist phentolamine, the facilitatory effect of vinpocetine was significantly lower ($P < 0.001$) than in its absence.

Inversely, the interaction of vinpocetine with phentolamine was also studied in such experiments in which vinpocetine was present throughout superfusion, whereas phentolamine was added to the superfusion medium before and during S_2 only. In the presence of vinpocetine

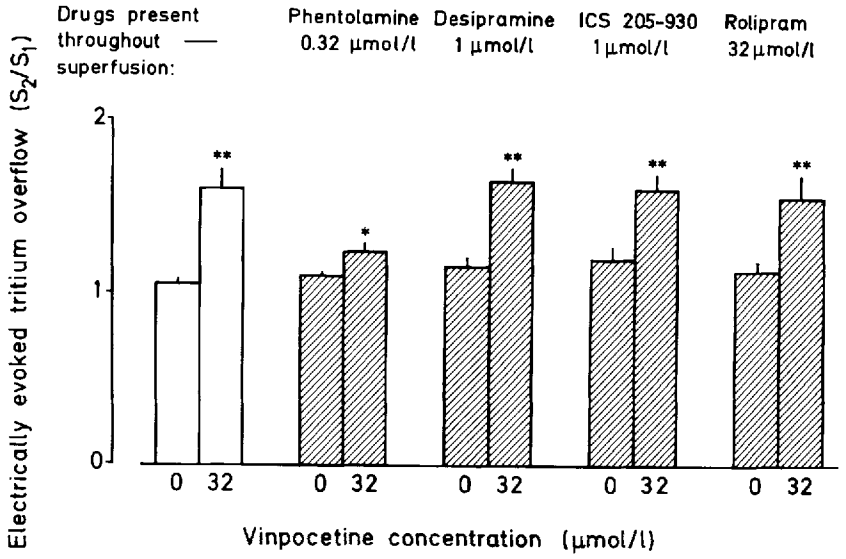

Fig. 2. Effect of vinpocetine on the electrically evoked ^{3}H overflow from superfused rat brain cortex slices preincubated with ^{3}H-noradrenaline, and interaction with drugs blocking α-adrenoceptors (phentolamine), noradrenaline uptake (desipramine), 5-HT$_3$ (M) receptors (ICS 205-930), and cAMP phosphodiesterase (rolipram). The superfusion fluid contained vinpocetine from the 62nd min onward and one of the interacting drugs throughout superfusion. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1 and S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). Means ± S.E.M. of 6–29 experiments. *P <0.05; **P <0.01.

32 μmol/liter, phentolamine 0.32 μmol/liter increased the electrically evoked overflow from 1.18 ± 0.05 (8 controls) to 2.96 ± 0.13 (n = 7; $P < 0.001$); the corresponding values, obtained in the absence of vinpocetine, were 1.03 ± 0.02 and 3.62 ± 0.18, respectively (n = 5–6; $P < 0.001$). The phentolamine-induced increase of the evoked overflow in the presence of vinpocetine (by 151%) is significantly lower ($P < 0.001$) than that in its absence (by 251%).

In the last series of experiments, the effect of vinpocetine or phentolamine (present throughout superfusion) on the concentration-response curve of noradrenaline (present before and during S_2) for its inhibitory effect on the evoked overflow was studied (Fig. 3). (Desipramine at 1 μmol/liter was included in the superfusion fluid of these experiments to avoid displacement of ^{3}H by noradrenaline.) Even at high concentrations, vinpocetine did not influence the concentration-response curve of noradrenaline, whereas phentolamine produced a shift to the right (pA_2 value: 7.74).

DISCUSSION

Vinpocetine facilitates the electrically evoked ^{3}H overflow from rat brain cortex slices preincubated with ^{3}H-noradrenaline. This effect occurs in the same concentration range and is of the same magnitude as the facilitatory effect of the drug on the electrically evoked ^{3}H-noradrenaline overflow from strips of rabbit pulmonary artery [Pauló et al., 1986]. Under the experimental conditions of the present study, the evoked overflow is tetrodotoxin-sensitive and calcium-dependent; it consists mainly of unmetabolized ^{3}H-noradrenaline [Taube et al., 1977], and thus represents quasiphysiological noradrenaline release.

Vinpocetine at concentrations of > 10 μmol/liter also accelerated the basal ^{3}H efflux. In general, interpretation of the evoked overflow is impeded under this condition, but one

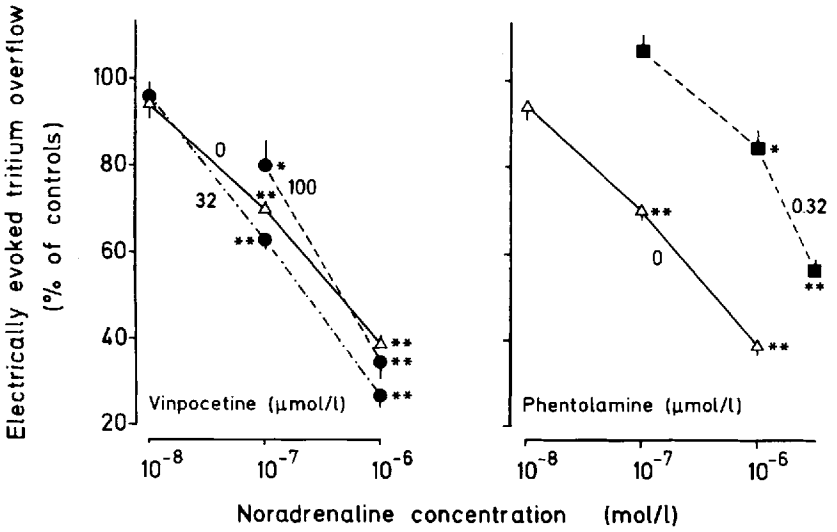

Fig. 3. Effect of unlabelled noradrenaline on the electrically evoked ^3H overflow from superfused rat brain cortex slices preincubated with ^3H-noradrenaline, and interaction with vinpocetine or phentolamine (reference drug). Noradrenaline was added to the superfusion medium from the 62nd min of superfusion onward, whereas desipramine 1 μmol/liter and, if relevant, vinpocetine or phentolamine were present throughout superfusion. Tritium overflow was evoked twice, after 40 and 90 min (S_1 and S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). Tritium overflow is given as percentage of the ratio S_2/S_1 in the corresponding controls (not shown). Means ± S.E.M. of 5–12 experiments. $^*P < 0.05$; $^{**}P < 0.01$.

should note that the facilitatory effect of vinpocetine on the evoked overflow occurred already at a concentration (10 μmol/liter) at which the basal efflux was not yet increased. Since vinpocetine is used for treatment of cerebral dysfunction of the elderly, its effect on the evoked overflow was also studied in slices of aged rats. However, there was no difference in terms of transmitter released from the brain slices obtained from rats of different ages.

In an attempt to explain the facilitatory effect of vinpocetine on noradrenaline release, the possibility was considered that the drug is a weak α_2-adrenoceptor antagonist. In brain slices, α_2-adrenoceptor antagonists, e.g., phentolamine, increase noradrenaline release due to interruption of the negative-feedback loop mediated by endogenous noradrenaline [Taube et al., 1977]. In the present experiments, when the presynaptic α_2-adrenoceptors were already blocked by phentolamine, the facilitatory effect of vinpocetine was strongly attenuated. This result, together with the finding that the facilitatory effect of phentolamine was moderately decreased in the presence of vinpocetine, argues in favour of α_2-adrenolytic properties of vinpocetine. However, in a more direct approach vinpocetine failed to influence the concentration-response curve of exogenous noradrenaline for its inhibitory effect on noradrenaline release. Nonetheless, the three findings may be reconciled. Thus, the facilitatory effect of a given α_2-adrenoceptor antagonist on noradrenaline release in brain slices occurs in a lower concentration range than its displacing effect on the concentration-response curve of exogenous noradrenaline (Schlicker et al., 1988b). This is related to the fact that the α_2-adrenoceptor antagonist under study competes not only with exogenous but also with an unknown amount of endogenous noradrenaline. (Due to the inhibition of noradrenaline uptake by desipramine to prevent displacement of tritium by exogenous noradrenaline, the concentration of endogenous noradrenaline is probably relatively high). Because of the poor solubility of vinpocetine, higher concentrations than 100 μmol/liter could not be examined, and, thus,

a definite decision whether or not the drug possesses α_2-adrenolytic properties in this model is not possible.

A weak antagonistic effect of vinpocetine at α_2-adrenoceptors would be in harmony with the finding of Lee and Geiger (personal communication) that the drug at a concentration of 1 μmol/liter slightly inhibits ^{3}H-rauwolscine binding to rat brain cortex membranes (data with higher concentrations of vinpocetine not available). Some evidence in favour of an α_2-adrenolytic property of vinpocetine comes also from the work of Pauló et al. [1986] on strips of rabbit pulmonary artery.

The facilitatory effect of vinpocetine may also be related to its reported inhibitory effect on phosphodiesterase in brain [Rosdy et al., 1976]. Activation of adenylate cyclase or inhibition of cAMP phosphodiesterase has been shown to increase noradrenaline release in rat brain slices [Schoffelmeer et al., 1985]. Moreover, vinpocetine, to a certain extent, resembles serotonin with respect to its chemical structure. Therefore, one may speculate that vinpocetine may activate facilitatory presynaptic serotonin receptors on the noradrenergic nerve fibres. The existence of such receptors has been shown in the rabbit hippocampus [Feuerstein and Hertting, 1986]. Finally, the facilitatory effect of vinpocetine may be due to inhibition of noradrenaline uptake. However, the failure of rolipram (an inhibitor of cAMP phosphodiesterase), ICS 205-930 (a 5-HT_3 [M] receptor antagonist), and desipramine, respectively, to affect the facilitatory effect of vinpocetine argues against an involvement of these mechanisms.

Irrespective of the mechanism, the question that arises is whether the facilitatory effect of vinpocetine on noradrenaline release contributes to its in vivo action. The plasma concentration in humans obtained with a standard dose of vinpocetine amounts to about 5 ng/ml ($\sim 10^{-8}$ mol/liter; Hammes and Weyhenmeyer, personal communication), and the concentration in brain tissue is of the same magnitude as in the plasma [findings on rats; Vereczkey et al., 1976]. Therefore, in humans, the effect of vinpocetine described in the present paper may be relevant, at best, after administration of excessive doses.

ACKNOWLEDGMENTS

The technical assistance of Mrs. D. Petri and Mrs. H. Plenker is gratefully acknowledged. We also thank Mrs. M. Hölkeskamp for typing the manuscript and the respective companies for the gifts of drugs.

REFERENCES

Feuerstein, T.J., and Hertting, G.: Serotonin (5-HT) enhances hippocampal noradrenaline (NA) release: Evidence for facilitatory 5-HT receptors within the CNS. Naunyn-Schmiedebergs Arch. Pharmacol. **333:**191–197, 1986.

Furchgott, R.F.: The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In Blaschko, H., Muscholl, E. (eds): "Handbook of Experimental Pharmacology," vol. XXXIII. New York: Springer, 1972, 283–335.

Pauló, T., Tóth, P.T., Nguyen, T.T., Forgács, L., Török, T.L., and Magyar, K.: [^{3}H] Noradrenaline-releasing action of vinpocetine in the isolated main pulmonary artery of the rabbit. J. Pharm. Pharmacol. **38:**668–673, 1986.

Rosdy, B., Balázs, M., and Szporny, L.: Biochemical effects of ethyl apovincaminate. Arzneimittel-Forschung **26:**1923–1926, 1976.

Schlicker, E., Classen, K., and Göthert, M.: Presynaptic serotonin receptors and α-adrenoceptors on central serotoninergic and noradrenergic neurones of normotensive and spontaneously hypertensive rats. J. Cardiovasc. Pharmacol., **11:**518–528, 1988a.

Schlicker, E., Molderings, G., Betz, R., and Göthert, M.: Antagonistic properties of Ru 249 69, a preferential 5-HT_1 receptor agonist, at presynaptic α-adrenoceptors of central and peripheral neurons. Pharmacol. Toxicol. (in press), 1988b.

Schoffelmeer, A.N.M., Wardeh, G., and Mulder, A.H.: Cyclic AMP facilitates the electrically evoked

release of radiolabelled noradrenaline, dopamine, and 5-hydroxytryptamine from rat brain slices. Naunyn-Schmiedeberg's Arch. Pharmacol. **330:**74–76, 1985.

Taube, H.D., Starke, K., and Borowski, E.: Presynaptic receptor systems on the noradrenergic neurones of rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. **299:**123–141, 1977.

Vereczkey, L., Zólyomi, G., and Szporny, L.: Pharmacokinetic data on tritium labelled ethyl apovincaminate. Arzneimittelforschung **26:**1929–1933, 1976.