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[^{3}H]Noradrenaline release evoked by selegiline ((−)-deprenyl) in the isolated main pulmonary artery of the rabbit*†

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High concentrations of selegiline((−)-deprenyl) (> 10^{-5} M) enhanced the nerve stimulation (2 Hz)-evoked release of [^{3}H]noradrenaline from the isolated main pulmonary artery of the rabbit. This facilitation of stimulation-evoked [^{3}H]noradrenaline release by selegiline was reduced by exogenous (−)-noradrenaline, an agonist of presynaptic α_2-adrenoceptors. This inhibitory action of (−)-noradrenaline was partly antagonized by yohimbine, a selective α_2-adrenoceptor blocker. When the stimulation-evoked [^{3}H]noradrenaline release had already been incresed by inhibition of Na^+-pump (K^+-free solution), selegiline further enhanced the nerve-evoked release of labelled neurotransmitter.

Selegiline ((−)-deprenyl; (−)-(*R*)-*N*-α-dimethyl-*N*-(prop-2-ynyl) phenethylamine HCl) is a selective blocker of the B form of monoamine oxidase (Knoll & Magyar 1972; Yang & Neff 1974). It strongly inhibits both the pharmacological action of tyramine (Knoll & Magyar 1972) and the dopamine uptake in striatum (Hársing et al 1979).

Selegiline is widely used as an adjunct of L-dopa in the treatment of Parkinson's disease. The present paper provides evidence that high concentrations of selegiline (> 10^{-5} M) increase the field stimulation evoked release of [^{3}H]noradrenaline from peripheral presynaptic nerve terminals when monoamine oxidase (MAO) and uptakes are inhibited.

MATERIALS AND METHODS

Rabbit main pulmonary artery

Rabbits of either sex (2–3 kg) were stunned by a blow on the head. The main pulmonary artery was dissected and placed into Krebs solution which contained pargyline ($1{\cdot}2\ 10^{-4}$ M) and was fully aerated with carbogen (5% CO_2 in O_2). The composition of the Krebs solution was (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 120·2; $H_2PO_4^-$, 1·2; HCO_3^-, 25·0;; SO_4^{2-}, 1·2; glucose, 11·5; Na_2EDTA, 0·03; ascorbic acid, 0·3. When K^+-free solution was used, KCl and KH_2PO_4 were omitted from the Krebs, and KH_2PO_4 was substituted by an equimolar concentration of NaH_2PO_4. In the latter case double distilled water was used for preparing the Krebs solution.

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Measurement of [^{3}H]noradrenaline release

Release of [^{3}H]noradrenaline [^{3}H]NA was measured by the method of Borowski et al (1977) and Török et al (1982). Briefly, after the preparation had been placed into the Krebs solution at 37 °C, 25 μl [^{3}H]NA was added to the incubation solution (final concentration of [^{3}H]NA: 2×10^{-6} M) for 45 min, pargyline was present. Subsequently the artery was suspended in a 2 ml organ bath and was superfused at a rate of 8 ml min^{-1} with 800 ml of medium containing cocaine (3×10^{-5} M) instead of pargyline. At the end of the washing period the flow rate was changed to 4 ml min^{-1} and corticosterone (5×10^{-5} M) was also added to the Krebs to block the extraneuronal uptake of noradrenaline. It has been shown that in the presence of cocaine and corticosterone 86% of liberated NA is unmetabolized (Endo et al 1977). On the basis of this assumption, and knowing the specific activity of [^{3}H]NA, we calculated the outflow of labelled neurotransmitter in pmol/3 min (Endo et al 1977).

To induce neurotransmitter release, the artery was stimulated (field stimulation) by electrical square-wave pulses (2 Hz, 1 ms, 60 V) for 3 min using platinum electrodes. The superfusate was fractionated and the radioactivity was determined in a Beckman liquid scintillation spectrometer. The stimulation evoked release of [^{3}H]NA was calculated by subtraction of the resting output immediately before stimulation from the release obtained during and up to 6 min after stimulation, and was expressed as pmol. In every experiment, 6 periods of field stimualtion were applied at 27 min intervals and 360 shocks at a frequency of 2 Hz were delivered during

each period. After three control periods, selegiline and/or (−)-NA was added to the perfusion solution 18 min before the period of stimulation. The effect of drugs was expressed as the ratio between the release of tritium [^{3}H]NA in pmol evoked by stimulation$_4$ and the release evoked by stimulation$_3$ (St_4/St_3). In control experiments the stimulation evoked release ratio St_4/St_3 proved to be 0·98 ± 0·02 (mean ± s.e.) in eight identical experiments.

Stimulation technique

Square wave pulses of 1 ms duration were delivered from a Grass S44 stimulator. Pulses were monitored on an oscilloscope.

Tension measurements

The same preparation was used for measurement of both release of radiolabel and isometric tension. The contractions of circular smooth muscle were measured with a strain gauge and recorded on a Servogor pen recorder.

Drugs

The drugs used were: (−)-[7-^{3}H]noradrenaline, specific activity: 37 Ci mmol^{-1} (Radiochemical Centre, Amersham, U.K.), pargyline hydrochloride (Serva), cocaine hydrochloride (Merck), corticosterone (Fluka), (−)-noradrenaline hydrochloride (Fluka), yohimbine hydrochloride (Koch-Light), selegiline ((−)-deprenyl hydrochloride Chinoin). The drugs were dissolved in Krebs solution. Corticosterone was dissolved in propylene glycol to a final concentration of 0·5 ml litre. All of the chemicals used to prepare Krebs solution were of analytical grade.

RESULTS

Facilitatory action of selegiline on stimulation evoked [^{3}H]noradrenaline release

Selegiline in a concentration of 10^{-6} M practically had no effect on the nerve stimulation evoked release of [^{3}H]NA. The ratio of evoked release was 1·06 ± 0·06 (mean ± s.e. of 4 experiments).

Selegiline, 10^{-5} M, increased the transmitter overflow in response to nerve stimulation (ratio: 1·26 ± 0·04; n = 4). When selegiline 10^{-4} M, was applied, the release of ^{3}H markedly increased with a ratio of 2·89 ± 0·15 (n = 8). However, the resting output of [^{3}H]NA did not change at any of the concentrations used. High concentration of selegiline (10^{-4} M) not only increased the transmitter overflow in response to nerve stimulation, but inhibited the stimulation-evoked contraction of smooth muscle by about 30–40% (Fig. 1). The pre- and postsynaptic effects

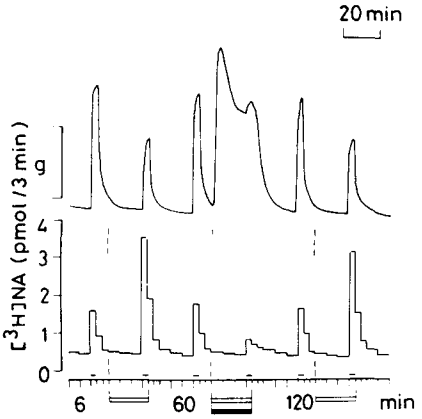

FIG. 1. Effect of selegiline (10^{-4} M) on [^{3}H]noradrenaline release from isolated pulmonary artery of the rabbit and the inhibitory action of (−)-noradrenaline (10^{-6} M). The outflow of [^{3}H]noradrenaline was expressed in pmol/3 min. Selegiline and noradrenaline were superfused from 18 min before stimulation$_4$ (St_4) onwards. Note, that noradrenaline reduced the selegiline-potentiated [^{3}H]noradrenaline release.
Upper panel: isometric contraction (in g as indicated) of circle smooth muscle.
Lower Panel: the outflow of [^{3}H]noradrenaline. Six periods of stimulation (2 Hz, 360 shocks, 3 min) took place at 27 min intervals. Dotted lines indicate the start of perfusion with drugs. The time scale is indicated. Stimulation periods are indicated by horizontal lines. Selegiline, 10^{-4} M (□); (−)-noradrenaline, 10^{-6} M(■).

of selegiline seem to be similar to those of phentolamine, an antagonist of α_2- and α_1-adrenoceptors (Langer 1979).

The inhibition of [^{3}H]noradrenaline releasing action of selegiline by activation of α_2-adrenoceptors

The presynaptic α_2-adrenoceptor plays a physiological role in the regulation of NA release (Langer 1974, 1977; Starke 1977). The selective α_2-receptor antagonist yohimbine (Starke et al 1975) in a concentration of 3×10^{-7} M increased the release of [^{3}H]NA in response to field stimulation (ratio: 3·98 ± 0·41; n = 6) indicating the existence of negative feedback inhibition at 2 Hz stimulation. This finding is in agreement with the observation of Borowski et al (1977).

Exogenous (−)-NA, which activates both α_2- and α_1-adrenoceptors, reduced the release of [^{3}H]NA during nerve stimulation, increased the tone of smooth muscle and inhibited the facilitation of NA release by selegiline (Figs 1, 2). Na, 10^{-6} M, which markedly inhibited the evoked release (ratio: 0·21 ± 0·12; n = 4) reduced the facilitatory action on [^{3}H]NA release of selegiline 10^{-4} M from 2·89 ± 0·15

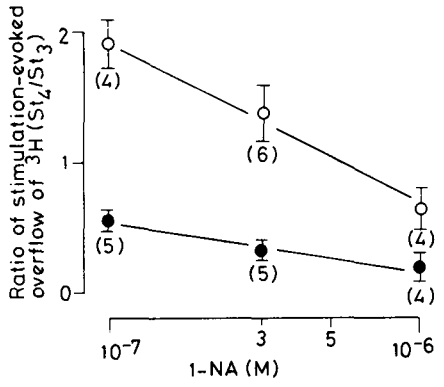

FIG. 2. Effect of (−)-noradrenaline (● — ●) and (−)-noradrenaline + selegiline 10^{-4} M (○ — ○) on stimulation (2 Hz for 3 min) evoked release of ^3H from the main pulmonary artery of the rabbit pre-incubated with [^3H]noradrenaline. Each artery was stimulated six times. Noradrenaline and noradrenaline plus selegiline was superfused from 18 min before stimulation$_4$ onwards. Results are expressed as the ratio between the overflow of tritium evoked by stimulation$_4$ (St_4) and that evoked by stimulation$_3$ (St_3). Means ± s.e.m. and the number of experiments are indicated.

Note, that (−)-noradrenaline inhibited both the stimulation evoked release of ^3H and the ^3H-releasing action of selegiline 10^{-4} M, in a concentration dependent manner ($P < 0.001$). In control experiments at 2 Hz (360 shocks) the overflow ratio (St_4/St_3) was 0·98 ± 0·02 (n = 8). The tritium outflow evoked by St_4 was 0·65 ± 0·01% of tissue tritium (75800 ± 350 counts min^{-1}, n = 8).

(n = 8) to 0·65 ± 0·16 (n = 4). The effect is significant at $P = 0.001$. NA inhibition of the facilitatory action of selegiline on release was partly antagonized by 3 × 10^{-7} M yohimbine (ratio: 1·04 ± 0·16; n = 5).

The [^3H]noradrenaline releasing effect of selegiline in Na^+-pump inhibited nerve fibres

It is known that the Na^+-pump plays a role in the regulation of neurotransmitter release (Paton et al 1971; Vizi 1972, 1977; Paton 1973; Baker & Crawford 1975; Nakazato et al 1978; Lorenz et al 1980). In Na^+-pump inhibited preparations, the internal Na^+ can exchange for external Ca^{2+} by the reversed Na/Ca exchange mechanism (Baker et al 1969; Baker 1972) resulting in transmitter release.

The question arises how selegiline acts on noradrenaline release when the Na^+-pump is already inhibited. If selegiline is capable of increasing the transmitter release in Na^+-pump-inhibited nerve terminals, it may increase the influx of Ca^{2+} by another mechanism. The Na^+-pump was inhibited by K^+-removal. In K^+-free medium the [^3H]NA release increased in response to nerve stimulation. The peak effect on nerve-evoked release was observed after 45 min of K^+-removal (second stimulation in K^+-free; ratio: 5·39 ± 0·54, n = 6). When selegiline, 10^{-4} M, was present, the ^3H overflow further increased (ratio: 8·76 ± 1·61; n = 4). The difference is significant at $P = 0.05$.

DISCUSSION

The present results provide evidence that high concentration of the MAO-B inhibitor selegiline is capable of increasing the release of [^3H]NA from MAO- and uptake inhibited sympathetic nerve fibres by nerve impulses.

In our experiments, selegiline not only facilitated transmitter release evoked by nerve stimulation but inhibited the contraction of smooth muscle. These effects are similar to that of phentolamine, a well known α-receptor blocking agent. Therefore, selegiline at >10^{-5} M may have α_2- and α_1-blocking actions.

The facilitation of nerve-evoked release of transmitter by selegiline was antagonized by activation of presynaptic α_2-receptors. The α_2-adrenoceptors inhibit the voltage-dependent Ca^{2+}-influx during the action potential (McAfee et al 1981) and thereby exert control over release of transmitter. Since Ca^{2+} inhibits the Na^+-pump (Na^+, K^+-ATPase; Skou 1965) and NA stimulates the enzyme (Gilbert et al 1975; Hexum 1977) it seems that the NA caused inhibition of NA release as a consequence of Na^+-pump activation. The exogenous NA caused depression of the facilitation of evoked transmitter release by selegiline and this was partly antagonized by the selective α_2-receptor blocker yohimbine.

During Na^+-pump inhibition, selegiline further enhanced the overflow of ^3H in response to field stimulation. Since in Na^+-pump-inhibited nerves the influx of calcium had already increased (Baker et al 1969; Baker 1972) it seems that selegiline may exert its transmitter releasing action by inhibition of nerve terminal Ca^{2+}-pump (uncoupled Ca^{2+}-extrusion; Schatzmann 1966, 1973) thereby causing additional influx of calcium and transmitter release.

In the treatment of Parkinson's disease the therapeutic blood concentration of selegiline should be about 10^{-6} M which does not significantly affect nerve-evoked transmitter release. However, during the expected accumulation of the drug in the vessels of the brain, the release of NA by the drug may not cause vasoconstriction since selegiline inhibits postsynaptic α_1-receptors at the same time.

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REFERENCES

Baker, P. F. (1972) Prog. Biophys. Mol. Biol. 24: 177–223

Baker, P. F., Blaustein, M. P., Hodgkin, A. L., Steinhardt, R. A. (1969) J. Physiol. (London) 200: 431–458

Baker, P. F., Crawford, A. C. (1975) Ibid. 247: 209–226

Borowski, E., Starke, K., Ehrl, H., Endo, T. (1977) Neurosci. 2: 285–296

Endo, T., Starke, K., Bangerter, A., Taube, H. D. (1977) Naunyn-Schmiedeberg's Arch. Pharmacol. 296: 229–247

Gilbert, I. C., Wyllie, M. G., Davison, D. V. (1975) Nature (London) 255: 237–238

Hársing, L. G. Jr., Magyar, K., Tekes, K., Vizi, E. S., Knoll, J. (1979) Pol. J. Pharmacol. Pharm. 31: 297–307

Hexum, T. D. (1977) Adv. Biochem. Psychopharmacol. 5: 393–408

Knoll, J., Magyar, K. (1982) Adv. Biochem. Psychopharmacol. 5: 393–408

Langer, S. Z. (1974) Biochem. Pharmacol. 23: 1793–1800

Langer, S. Z. (1977) Br. J. Pharmacol. 60: 481–497

Langer, S. Z. (1979) in: The Release of Catecholamines from Adrenergic Neurons. Paton, D. M. (ed.) Pergamon Press, Oxford, pp 59–85

Lorenz, R. R., Powis, D. A., Vanhoutte, P. M., Shepherd, J. T. (1980) Circ. Res. 47: 845–854

McAfee, D. A., Henon, B. K., Horn, J. P., Yarowsky, P. (1981) Fed. Proc. Fed. Am. Soc. Exp. Biol. 40: 2246–2249

Nakazato, Y., Ohga, A., Onoda, Y. (1978) J. Physiol. (London) 278: 45–54

Paton, D. M. (1973) Br. J. Pharmacol. 49: 614–627

Paton, W. D. M., Vizi, E. S., Zar, M. A. (1971) J. Physiol. (London) 215: 819–848

Schatzmann, H. J. (1966) Experientia 22: 364–368

Schatzmann, H. J. (1973) J. Physiol. (London) 235: 551–569

Skou, J. C. (1965) Physiol. Rev. 45: 596–617

Starke, K. (1977) Rev. Physiol. Biochem. Pharmacol. 77: 1–124

Starke, K., Borowski, E., Endo, T. (1975) Eu. J. Pharmacol. 34: 385–388

Török, T. L. Rubányi, G., Vizi, E. S., Magyar, K. (1982) Eu. J. Pharmacol. 84: 93–98

Vizi, E. S. (1972) J. Physiol. (London) 226: 95–117

Vizi, E. S. (1977) Ibid. 267: 261–280

Yang, H.-Y. T., Neff, N. H. (1974) J. Pharmacol. Exp. Ther. 189: 733–740