J. Pharm. Pharmacol. 1980, 32: 92–96 Received June 5, 1979

Facilitatory effect of nicotine on adrenergic neuroeffector transmission in the isolated ear artery of the rabbit

CATHERINE SARANTOS-LASKA*, MARIAN W. MCCULLOCH AND MICHAEL J. RAND

Department of Pharmacology, University of Melbourne, Parkville, Victoria, 3052, Australia

The effects of nicotine were studied on perfusion pressure and vasoconstrictor responses to sympathetic nerve stimulation in the isolated ear artery of the rabbit. Infusions of nicotine (50 μM) produced a transient increase in perfusion pressure and potentiated responses to nerve stimulation; these effects of nicotine were unaffected by atropine (0·3 μM) and abolished or significantly reduced respectively by hexamethonium (300 μM) or mecamylamine (1 μM). In experiments with ear arteries previously labelled with [^{3}H] noradrenaline an infusion of nicotine (50 μM) produced a transient increase in tritium efflux and the potentiation of responses to nerve stimulation in the presence of nicotine was accompanied by a statistically significant increase in stimulation-induced tritium efflux; these effects of nicotine were abolished by hexamethonium (300 μM) or mecamylamine (1 μM).

It is now well known that nicotine releases noradrenaline from sympathetic nerve terminals. This effect of nicotine has been shown in the isolated heart of the rabbit (Lindmar et al 1968; Löffelholz 1970a), in rabbit ear arteries (Burn & Rand 1957, 1958; Burn et al 1959), rabbit pulmonary artery (Su & Bevan 1970), perfused guinea-pig heart (Westfall & Brasted 1972) and superfused brain slices (Westfall 1974). The noradrenaline releasing action of nicotine has further been demonstrated in experiments using reserpine (Gillespie & Mackenna 1960; Su & Bevan 1970; Steinsland & Furchgott 1975a), 6-hydroxydopamine (Westfall 1971), denervation (Ferry 1966), adrenergic neuron blocking drugs and phenoxybenzamine (Steinsland & Furchgott 1975a).

There is evidence that the receptors involved have the pharmacological characteristics of nicotinic receptors, as the releasing action of nicotine is blocked by hexamethonium (Westfall & Brasted 1972, 1974) and the contractile response to nicotine is blocked by hexamethonium, mecamylamine and tetraethyl ammonium (Steinsland & Furchgott 1975a).

The noradrenaline releasing action of nicotine is very short-lasting, despite the continued presence of nicotine and has been described as "explosive" (Löffelholz 1970a). However, responses to nerve stimulation elicited after the cessation of release of noradrenaline by nicotine and in the continued presence of nicotine are unimpared (Löffelholtz 1970b) and in certain tissues, nicotine has been found to potentiate the responses to sympathetic nerve stimulation (Nedergaard & Bevan 1969; Su & Bevan 1970; Steinsland & Furchgott 1975b; Nedergaard & Schrold 1977). In this study, the effects of nicotine on responses and transmitter release have been investigated using the isolated ear artery of the rabbit.

* Correspondence.

MATERIALS AND METHODS

Rabbits were killed by cervical dislocation and the central ear artery segments were set up according to the method of Allen et al (1973a). The segment was perfused-superfused with Krebs-Henseleit solution at a constant flow rate of 4 ml min^{-1}, maintained by a peristaltic tubing pump (Watson Marlow H.R. flow inducer). The Krebs-Henseleit solution was of the following composition: (mmol litre^{-1}): NaCl, 118; KCl, 4·7; $NaHCO_3$, 25·0; $MgSO_4$, 0·45; KH_2PO_4, 1·03; $CaCl_2$, 2·5 and D-(+)-glucose, 11·1. Disodium edetate (0·067 mmol litre^{-1}) was added to the Krebs-Henseleit solution to retard oxidation of noradrenaline. The solution was equilibrated at 37°C with a mixture of 5% carbon dioxide in oxygen. The perfusion pressure was measured with a Statham P23Db pressure transducer and recorded with a Tohshin Electron TO2N2H potentiometric pen recorder. Periarterial sympathetic nerves were stimulated using bipolar circular platinum electrodes and by applying monophasic square wave pulses of 1 ms duration, supramaximal voltage (40 V, which gave a p.d. across the electrodes of 12 V) at 10 Hz for 10 s at 3 min intervals using a Grass S44 stimulator.

In other experiments the artery segments were incubated with (−)-[^{3}H]noradrenaline (10 μCi ml^{-1}, 0·3–0·4 μg ml^{-1}) for 1 h in a separate bath, remounted on the perfusion-superfusion apparatus and washed with (−)-[^{3}H]noradrenaline-free Krebs-

Henseleit solution for 90 min to remove any loosely bound noradrenaline and metabolites. The arteries were stimulated (10 Hz, 1 ms for 10 s) for 7 periods at 4 min intervals. Samples of the perfusate-superfusate were collected over 1 min periods throughout each experiment, after two resting samples before the first period of stimulation had been collected.

For estimation of total tritium efflux, a 1 ml aliquot of each sample was placed in a collecting vial containing 10 ml of a scintillation solution and two drops of 6M HCl. The scintillation solution consisted of 5·5 g of 2,5-diphenyloxazole (PPO), 0·1 g of 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP) and 33 ml of Triton-X100 made up to one litre with toluene. The vials were counted in a Packard Tricarb 3380 liquid scintillation spectrometer for 50 min. Corrections for counting efficiency were made using an internal reference standard ([^{3}H]-n-hexadecane).

The stimulation-induced efflux at the first stimulation period was calculated by subtracting the average resting level of radioactivity before stimulation from the total increase during and after stimulation. The same basic calculation procedure was used for the six subsequent stimulation periods, except that the resting value was taken from the sample immediately preceding stimulation. Stimulation-induced effluxes for periods 2 to 7 were calculated as a percentage of that for the first period. The total radioactivity of samples was calculated as disintegrations per minute (d min^{-1}) per sample.

Drugs used were: atropine sulphate (BDH); hexamethonium bromide (Koch-Light); mecamylamine hydrochloride (Merck, Sharp and Dohme); nicotine hydrogen (+)-tartrate (BDH); (−)-[^{3}H]-noradrenaline (3·8 Ci mmol^{-1}, New England Nuclear Corporation). All drugs were dissolved in distilled water and the concentrations are expressed in terms of the respective salts. Drugs were introduced into the perfusing solution from a motor driven syringe (Braun-Melsungen UNITAR I). The statistical analysis of results was carried out using Student's *t*-test.

RESULTS

Effect of nicotine on perfusion pressure and vasoconstrictor responses to sympathetic nerve stimulation in the isolated ear artery of the rabbit

An infusion of nicotine (50 μM) produced an increase

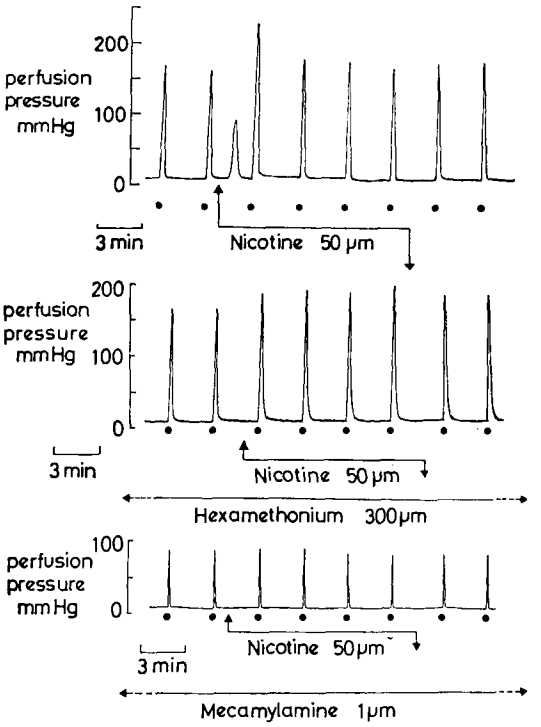

FIG. 1. Effects of nicotine infusion (50 μM) alone (top), in the presence of hexamethonium (300 μM) (middle) and in the presence of mecamylamine (1 μM) (bottom) on perfusion pressure and vasocor trictor responses to sympathetic nerve stimulation (1 ms pulses at 10 Hz for 10 s indicated by ● every 3 min) in the isolated ear artery of the rabbit.

Table 1. The effects of nicotine alone and nicotine in the presence of hexamethonium, mecamylamine and atropine on responses (R) to electrical stimulation (10 Hz, 1 ms for 10 s) of the rabbit ear artery at 3 min intervals (R_1 to R_8). The responses are expressed as percent of control (mean of R_1 and R_2) in absence of nicotine. Nicotine infusion commenced 2 min before to R_3 and was present for R_3–R_8. Infusions of the three antagonists were applied 15 min before nicotine and were present throughout the experiments. n is the number of experiments.

	n	R_1,R_2	R_3	R_4	R_5	R_6	R_7	R_8
Nicotine (50 μM)	30	100	127·4±11·0*	105·3±12·3	102·9±12·8	103·4±12·4	99·5±12·6	100·0±12·6
Nicotine (50 μM) in presence of hexamethonium (300 μM)	10	100	104·4±10·8†	99·5±10·7	103·4±14·5	101·7±14·7	96·6±16·0	97·6±15·7
Nicotine (50 μM) in presence of mecamylamine (1 μM)	15	100	110·2±14·5†	99·5±10·7	101·6±11·0	99·5±10·7	99·5±11·5	95·6±11·0
Nicotine (50 μM) in presence of atropine (0·3 μM)	6	100	135·1±10·2*	104·6±2·7	103·6±4·1	107·5±4·5	102·6±5·5	102·7±4·6

* $P<0·05$ compared with control (R_1,R_2) by paired Student's *t*-test.
† $P<0·05$ compared with effect of nicotine alone (R_3) by unpaired Student's *t*-test.
‡ Mecamylamine, hexamethonium and atropine had no significant effect on responses to sympathetic nerve stimulation.

in perfusion pressure in the ear artery which rapidly returned to pre-infusion level in the continued presence of nicotine (Fig. 1). In addition, this concentration of nicotine, applied 2 min before nerve stimulation, significantly potentiated the response to stimulation (Fig. 1, Table 1). This potentiation could also be demonstrated with much lower concentrations of nicotine (0·1 μM), which did not produce a direct vasoconstrictor response. When the time interval between the start of nicotine infusion and sympathetic nerve stimulation was greater than 6 min, no potentiation of the response to sympathetic nerve stimulation was observed.

Effect of nicotine in the presence of hexamethonium, mecamylamine and atropine

Infusion of each of the antagonists commenced 15 min before nicotine and continued throughout the experiment. Hexamethonium (300 μM) and mecamylamine (1 μM) abolished the vasoconstrictor response to nicotine and significantly reduced the potentiation of responses to sympathetic nerve stimulation by nicotine (50 μM) (Fig. 1 Table 1). On completion of the experiment, reapplication of nicotine readily produced the same effects after cessation of hexamethonium infusion. With mecamylamine however, at least 80 min elapsed after removal of mecamylamine before the effects of nicotine could be repeated.

Atropine (0·3 μM) did not affect the vasoconstrictor responses to or the potentiation of responses to sympathetic nerve stimulation by nicotine (50 μM) (Table 1).

Effect of nicotine on tritium efflux

An infusion of nicotine (50 μM), in the absence of nerve stimulation, produced a short-lived increase in ^{3}H-efflux (Fig. 2a).

In control experiments the mean stimulation-induced efflux of tritium in the third period of stimulation was 70·0% (s.e.m. = 12·1, n = 4) of that in the first stimulation period (Fig. 3a). Nicotine (50 μM) infused 1 min before the third stimulation period produced a slight increase in the resting efflux and a marked increase in the stimulation-induced efflux of tritium in the third period of stimulation (mean = 195·42% of S_1, s.e.m. = 11·98, n = 5); in the continued presence of nicotine subsequent stimulation-induced effluxes were not different from control (Figs 2 lower graph, 3b).

Effects of hexamethonium and mecamylamine on the potentiation of stimulation-induced efflux by nicotine

Both hexamethonium (300 μM) and mecamylamine

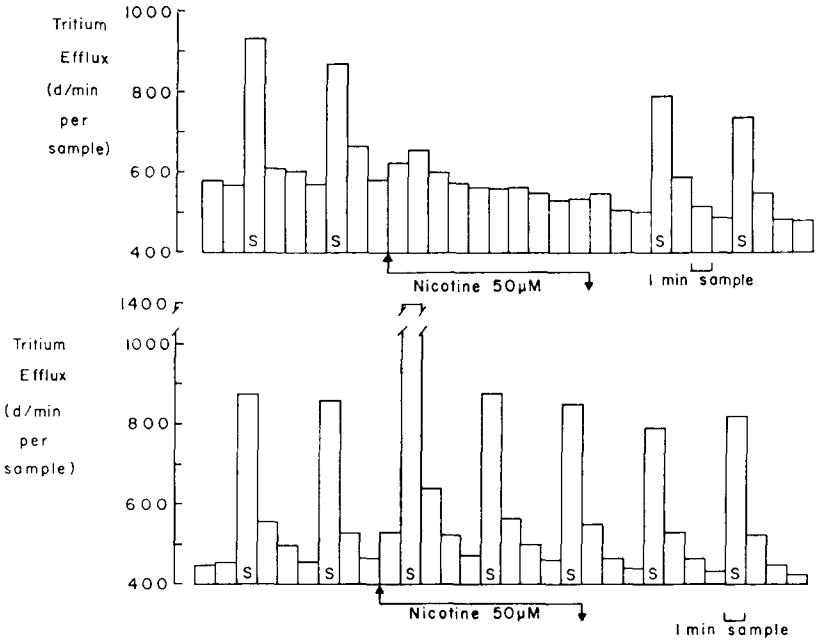

FIG. 2. Effects of nicotine infusion (50 μM) on tritium efflux in the isolated ear artery of the rabbit previously incubated with [^{3}H]noradrenaline. Sympathetic nerve stimulation (S) (1 ms pulses; 10 Hz; 10 s; every 4 min) was applied before and after nicotine infusion (top) and during the nicotine infusion (bottom). 1 min samples of perfusion-superfusion solution were collected throughout the experiment and the radioactivity expressed as disintegrations per min (d min^{-1}) per sample.

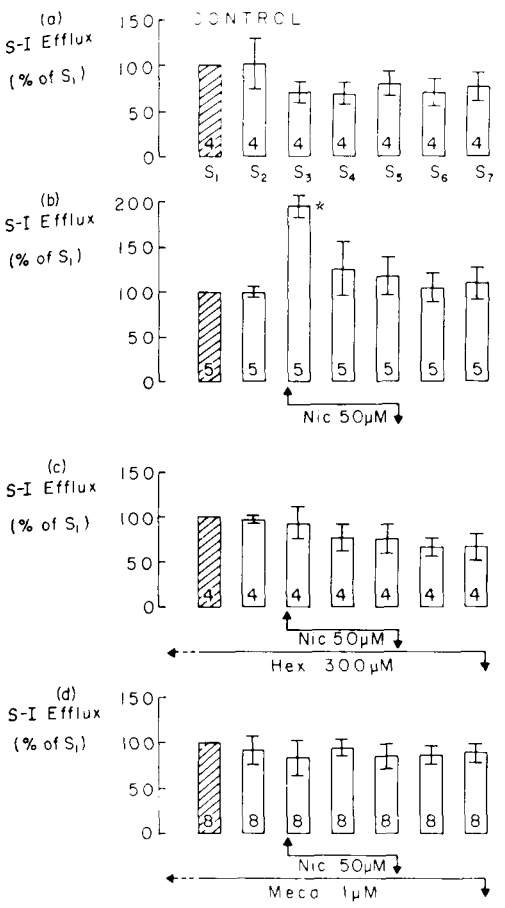

FIG. 3. (a) Mean stimulation-induced (S-I) effluxes in control experiments. S_1 to S_7 represent seven stimulation periods at 4 min intervals (1 ms pulses; 10 Hz; for 10 s). The results are expressed as S-I efflux as a percent of the S-I efflux in the first stimulation period (S_1). The vertical bars represent standard errors of the mean. The number of experiments is indicated in each column.

(b) Effects of nicotine (Nic) infusion (50 μM) during S_2 to S_5. The asterisk denotes a significant difference in S-I efflux from control ($P < 0.05$, Student's paired t-test).

(c) Effects of nicotine infusion (50 μM) during S_3 to S_5 in the presence of hexamethonium (Hex.) (300 μM).

(d) Effects of nicotine infusion (50 μM) during S_3 to S_5 in the presence of mecamylamine (Meca) (1 μM).

(1 μM) infused 15 min before nicotine and during the remainder of the experiment significantly blocked the potentiating effect of nicotine (50 μM) on stimulation-induced efflux, without affecting the first two control periods of stimulation (Fig. 3c,d).

DISCUSSION

The ability of nicotine to cause a transient release of noradrenaline from sympathetic nerve terminals has been confirmed in these experiments. Nicotine also caused a potentiation of responses to sympathetic nerve stimulation. It is unlikely that this potentiation is due to blockade of neuronal uptake of noradrenaline, since Allen et al (1973b) have shown nicotine to have only a slight inhibitory effect on the uptake of noradrenaline in guinea-pig atria and Westfall & Brasted (1972) have shown that nicotine does not inhibit the uptake of [^{3}H]noradrenaline by guinea-pig heart. Furthermore, nicotine does not affect responses to noradrenaline (Nedergaard & Bevan 1969; Nedergaard & Schrold 1977).

The time interval between the commencement of infusion of nicotine and the electrical stimulation seems to be of importance for the potentiating action. It was found that if this time interval was greater than 6 min, no potentiation of the response to nerve stimulation was observed. Similarly with the pulmonary artery (Nedergaard & Bevan 1969) no potentiation was observed after exposure to nicotine for a period of at least 20 min before stimulation, whereas if the artery was stimulated at 10 min intervals, a single concentration of nicotine (10 μM) potentiated responses to sympathetic nerve stimulation. In the experiments reported here, potentiation of only the response to nerve stimulation immediately following the commencement of infusion of nicotine was seen. Thus it appears that the interaction between nicotine and nerve stimulation resulting in its potentiation is very short-lived and perhaps also subject to desensitization. The term 'desensitization' was used by Steinsland & Furchgott (1975b) to describe the short-lasting vasoconstrictor response to nicotine in the rabbit ear artery.

The effects of nicotine were studied in a range of concentrations (0·1 μM to 1000 μM) and 50 μM was chosen since reproducible effects were observed with this concentration.

Atropine affected neither the vasoconstrictor response to nicotine nor the potentiation of the response to nerve stimulation immediately following nicotine infusion, indicating that muscarinic receptors are not involved. Hexamethonium, however, blocked the vasoconstrictor response to nicotine and significantly reduced the potentiation by nicotine of responses to nerve stimulation. Thus inhibitory effects of hexamethonium were readily reversible. Mecamylamine also blocked both the vasoconstrictor response and the potentiation of responses to nerve stimulation by nicotine. However, the inhibitory effects of mecamylamine were less readily reversed.

These results indicate that nicotine has two separate and independent effects in this tissue. Firstly, the

short-lived contractile response of the artery and secondly, the potentiation of the response to sympathetic nerve stimulation. Both effects of nicotine are blocked by nicotinic receptor antagonists and it would appear that the nicotinic receptors involved are on the outer surface of the adrenergic nerve terminal, as it is unlikely that the bis-quaternary hexamethonium is able to enter the neuron (Nedergaard & Schrold 1977) (whereas mecamylamine can probably enter freely). These results are in agreement with those of Nedergaard & Schrold (1977) in the rabbit pulmonary artery.

Conflicting evidence appears in the literature as to whether it is necessary for nicotine to enter the nerve terminal before it can exert its effect. Su & Bevan (1970) suggested that the action of nicotine may require an intact noradrenaline uptake mechanism since the effect of nicotine was blocked by the noradrenaline uptake blocking drugs cocaine, desipramine and phenoxybenzamine. They also studied the uptake of labelled nicotine and found that there was an initial uptake of nicotine in the region of the terminal sympathetic plexus which was reduced by phenoxybenzamine, cocaine or desipramine (Bevan & Su 1972). However, considerable evidence has been provided by Nedergaard & Bevan (1971), Westfall & Brasted (1972) and Nedergaard & Schrold (1977) supporting the view that nicotine acts by stimulation of a nicotinic receptor at the nerve terminal and that the action of nicotine is independent of an intact noradrenaline uptake system. The present results tend to support the latter view, both in the case of vasoconstrictor responses to nicotine and the potentiation of the response to nerve stimulation.

Measurement of transmitter release in these experiments correlated well with the responses of the arteries. The potentiating effect of nicotine on the response to nerve stimulation was accompanied by a significant increase in tritium release. It was thought that the increase in stimulation-induced tritium release by nicotine might be a result of the releasing action of nicotine itself. However, Fig. 2 demonstrates that this release is not large enough to account for the significant increase in stimulation-induced efflux, indicating that, although the same receptors are involved, these two mechanisms are independent. Furthermore, potentiation of the response to sympathetic nerve stimulation immediately following nicotine infusion was seen at concentrations of nicotine too low to produce a vasoconstrictor response and if the concentration of nicotine was high enough to cause vasoconstriction, this effect had declined before potentiation of the responses to sympathetic nerve stimulation was seen.

Mecamylamine and hexamethonium blocked both the nicotine-induced potentiation of sympathetic nerve responses and the increase in stimulation-induced efflux. It thus appears that in addition to its well documented sympathomimetic action, nicotine can also exert a facilitatory action on responses to nerve stimulation through an action on nicotinic receptors. The suggestion of increased Ca^{2+} uptake by the neurons made by Nedergaard & Schrold (1977) is a possible mechanism.

REFERENCES

Allen, G. S., Rand, M. J., Story, D. F. (1973a) Cardiovasc. Res. 7: 423–428

Allen, G. S., Rand, M. J., Story, D. F. (1973b) Br. J. Pharmacol. 47: 179–180

Bevan, J. A., Su, C. (1972) J. Pharmacol. Exp. Ther. 182: 419–426

Burn, J. H., Rand, M. J. (1957) Lancet 2: 1097

Burn, J. H., Rand, M. J. (1958) Br. Med. J. 1: 903–908

Burn, J. H., Leach, E. H., Rand, M. J., Thompson, J. W. (1959) J. Physiol. 148: 332–352

Ferry, C. B. (1966) Physiol. Rev. 46: 420–456

Gillespie, J. S., Mackenna, B. R. (1960) J. Physiol. (London) 152: 191–205

Lindmar, R., Löffelholz, K., Muscholl, E. (1968) Br. J. Pharmacol. 32: 280–294

Löffelholz, K. (1970a) Naunyn Schmiedeberg's Arch. Pharmacol. 267: 49–63

Löffelholz, K. (1970b) Ibid. 267: 64–73

Nedergaard, O. A., Bevan, J. A. (1969) J. Pharmacol. Exp. Ther. 168: 127–136

Nedergaard, O. A., Bevan, J. A. (1971) in: Bevan, J. A., Furchgott, R. F., Maxwell, R. A., Somlyo, A. P. (eds) Physiology and Pharmacology of Vascular Neuroeffector Systems, S. Karger, Basel p22

Nedergaard, O. A., Schrold, J. (1977) Eur. J. Pharmacol. 42: 315–329

Steinsland, O. S., Furchgott, R. F. (1975a) J. Pharmacol. Exp. Ther. 193: 128–137

Steinsland, O. S., Furchgott, R. F. (1975b) Ibid. 193: 138–148

Su, C., Bevan, J. A. (1970) Ibid. 175: 533–540

Westfall, T. C. (1971) Pharmacologist 13: 229

Westfall, T. C. (1974) in: Usdin, E., Snyder, S. (eds) Frontiers in Catecholamine Research, Pergamon Press: New York pp 617–618

Westfall, T. C., Brasted, M. (1972) J. Pharmacol. Exp. Ther. 182: 409–418

Westfall, T. C., Brasted, M. (1974) Ibid. 189: 659–664