British Journal of Pharmacology (1997) 120, 298–304

Contribution of adrenal hormones to nicotine-induced inhibition of synovial plasma extravasation in the rat

*Frederick Jia-Pei Miao, *,†Neal L. Benowitz, *,**Philip H. Heller & *,†,‡,¶,[1]Jon D. Levine

*Departments of Medicine (Divisions of †Clinical Pharmacology and Experimental Therapeutics, and ‡Rheumatology and Clinical Immunology); ¶Anatomy and Oral and Maxillofacial Surgery, University of California at San Francisco Schools of Medicine and Dentistry, San Francisco, CA 94143-0452; and **Kaiser Foundation Hospital, Hayward, CA 94545, U.S.A.

1 In this study, we examined the mechanism(s) by which s.c. nicotine inhibits synovial plasma extravasation. We found that nicotine dose-dependently inhibited bradykinin (BK)- and platelet activating factor (PAF)-induced plasma extravasation.

2 The effect of nicotine on both BK- and PAF-induced plasma extravasation was attenuated by adrenal medullectomy. ICI-118,551 (a selective β_2-adrenoceptor blocker) (30 μg ml^{-1}, intra-articularly) significantly attenuated the inhibitory action of high-dose (1 mg kg^{-1}) nicotine on BK-induced plasma extravasation without affecting the inhibition by low- (0.01 μg kg^{-1}) dose nicotine or that on PAF-induced plasma extravasation by nicotine at any dose. This suggested that β_2-adrenoceptors mediate the inhibitory actions of high-dose, but not low-dose, nicotine. We also found that systemic naloxone (an opioid receptor antagonist) (two hourly injections of 1 mg kg^{-1}, i.p.) attenuated the inhibitory action produced by all doses of nicotine on BK- or PAF-induced plasma extravasation, suggesting the contribution of endogenous opioids.

3 RU-38,486 (a glucocorticoid receptor antagonist) (30 mg kg^{-1}, s.c.) and metyrapone (a glucocorticoid synthesis inhibitor) (two hourly injections of 100 mg kg^{-1}, i.p.) both attenuated the action of high-dose nicotine without affecting that of low-dose nicotine.

4 Spinal mecamylamine (a nicotinic receptor antagonist) (0.025 mg kg^{-1}, intrathecally, i.t.) attenuated the action of high-dose, but not low-dose, nicotine, suggesting that part of the action of high-dose nicotine is mediated by spinal nicotinic receptors.

5 Combined treatment with ICI-118,551, naloxone and RU-38,486 attenuated the action of low-dose nicotine by an amount similar to that produced by naloxone alone but produced significantly greater attenuation of the effect of high-dose nicotine when compared to the action of any of the three antagonists alone.

Keywords: Nicotine; microvascular permeability; adrenal medulla; adrenal cortex; spinal nervous system; inflammation

Introduction

It has been shown that nicotine activates primary afferent nociceptors (Steen & Reeh, 1993) and that nicotinic cholinergic circuitry plays a role in nociceptive pathways (e.g., Rogers & Iwamoto, 1993; Khan *et al*., 1994). Since pain is a component of the inflammatory process (dolore) and since activation of primary nociceptors also attenuates microvascular permeability (Green *et al*., 1995, and unpublished observation), via neural and endocrine circuits (Green *et al*., 1995), we hypothesized that activating nociceptive pathways, through stimulation of the nicotinic cholinergic circuitry may also suppress synovial plasma extravasation and that such an action is mediated by the same neural/endocrine circuit.

Recently, we found that nicotine (s.c.), starting at doses as low as 0.01 μg kg^{-1}, inhibited the plasma extravasation induced by the inflammatory mediator, bradykinin (BK) (Miao *et al*., 1992a), in part via an adrenal medulla-dependent mechanism (Miao *et al*., 1992a,b). The factors mediating this inhibitory action have not been clarified. Since nicotine can release adrenaline from the adrenal medulla (Cryer *et al*., 1976; McKay & Trent-Sanchez, 1990) and since activation of β_2-adrenoceptors in the knee joint, by salbutamol, inhibits BK-induced plasma extravasation (Coderre *et al*., 1990; 1991), it has been suggested that the inhibitory action of nicotine on BK-induced plasma extravasation is mediated by adrenaline (Miao *et al*., 1992a,b). However, no increase in plasma adrenaline was detected following administration of nicotine at doses below 1 mg kg^{-1}, s.c. by use of a very sensitive high performance liquid chromatographic (h.p.l.c.) assay (unpublished data). Since β_2-adrenoceptors occur presynaptically on sympathetic postganglionic neurone terminals, we compared the effect of nicotine on the sympathetic postganglionic neurone terminal-dependent plasma extravasation induced by a relatively low dose of BK (Miao *et al*., 1996a,b) with that induced by platelet activating factor (PAF), an inflammatory mediator that stimulates plasma extravasation independently of sympathetic mechanisms (Green *et al*., 1993a; Miao *et al*., 1996a,b).

In addition to adrenaline, other adrenal medullary hormones (e.g., enkephalins and neuropeptide Y) which can be released by nicotine (Hexum & Rusett, 1989; Stachowiak *et al*., 1990; Wilson, 1991) also inhibit synovial plasma extravasation (Green *et al*., 1993b). Finally, corticosterone, an adrenal cortical hormone, can be released by nicotine (Pomerleau & Pomerleau, 1990; Donnerer & Lembeck, 1990; Krueger *et al*., 1991; Stalke *et al*., 1992). Although its action on synovial plasma extravasation is not known, glucocorticoid inhibits plasma extravasation in many tissues (Tsurufuji *et al*., 1980; Seghaye *et al*., 1986; Brattsand *et al*., 1991). Therefore, we tested the hypothesis that these adrenal hormones also contribute to the inhibitory effect of nicotine.

Finally, since nicotine can release glucocorticoids via an action on the central nervous system (Krueger *et al*., 1991; Matta *et al*., 1990; 1993) and as spinal pathways mediate part of the inhibitory effect of nicotine on synovial plasma extravasation (Miao *et al*., 1994), we also studied the role of spinal nicotinic receptors.

[1]Author for correspondence at: Department of Medicine, University of California at San Francisco, School of Medicine, Box 0452, San Francisco, CA 94143-0452, U.S.A.

Methods

The experiments were performed on male Sprague-Dawley rats (300–400 g, from Bantin and Kingman, Fremont, CA) that were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.) (Anthony Products Co., Arcadia, CA).

Perfusion of the knee joint

Knee joint perfusion was performed, as previously described (Coderre *et al*., 1989). In brief, after incision of the skin and connective tissue overlying the anterior aspect of the knee, Evans blue dye (50 mg kg^{-1}) was administered i.v. in the saphenous vein via a 30-gauge hypodermic needle which was then withdrawn from the vein. Ten minutes after injection of the dye, a 30-gauge needle was inserted into the cavity of the knee joint for the infusion of fluid (250 μl min^{-1}, controlled by a syringe pump from Sage Instruments, Model 351, Cambridge, MA). After infusion of an initial volume of 100–200 μl of vehicle, a second needle (25-gauge) was inserted into the knee joint, approximately 3 mm from the inflow needle. This second needle served as an outflow cannula. Fluid was withdrawn from the joint through the outflow cannula by a second syringe pump. The perfusing fluid was infused and withdrawn at a constant rate of 250 μl min^{-1}. Perfusate samples were collected every 5 min for a period of 85 min. Samples were analysed for the amount of Evans blue dye by spectrophotometric measurement of absorbance at 620 nm. The absorbance at this wavelength is linearly related to dye concentration (Carr & Wilhelm, 1964).

After a baseline perfusion period of 15 min with vehicle (normal saline), plasma extravasation into the knee joint was stimulated by adding BK (160 ng ml^{-1} or approximately 0.15 μM) or PAF (52.4 ng ml^{-1} or 0.1 μM) to the perfusion fluid. These doses of BK and PAF have previously been demonstrated to be ED_{50} values on their respective dose-response curves (Green *et al*., 1993a). Of note, the concentration of BK in various inflamed tissues is in the range of 50 nM to 0.1 μM (Swift *et al*., 1993; Hargreaves *et al*., 1993 and Hargreaves, personal communication) while that of PAF is approximately 1.7 μM (Will *et al*., 1991; Appleyard & Hillier, 1995). A similar concentration of BK has been used as a component of so-called 'inflammatory soup' (e.g., Steen *et al*., 1995). Experiments involving both knee joints in the same rat were performed simultaneously.

Drug administration protocols

Nicotine To evaluate the effect of nicotine on synovial plasma extravasation induced by BK and PAF, we administered nicotine s.c. in the neck. Nicotine was prepared in 0.1 ml normal saline. Two doses of nicotine were administered in individual groups of animals (0.01 μg kg^{-1} and 1 mg kg^{-1}). Since the inhibitory effects of these doses were similar to those produced in a cumulative dose-response curve (data not shown), they were selected to allow examinion of the mechanism of action of nicotine at different doses. Since the effect of a mid-dose (3 μg kg^{-1}) of nicotine (data not shown) was similar to that induced by low-dose nicotine, only the effect of the latter dose is presented. Synovial plasma extravasation was sampled every 5 min (starting 30 min before administration of nicotine until 40 min after injection of nicotine). The time interval between nicotine injection and the maximum inhibition of plasma extravasation by nicotine was 30 min (Miao *et al*., 1992a).

ICI-118,551 To study the contribution of adrenaline to the nicotinic inhibition of BK- or PAF-induced plasma extravasation, we co-perfused ICI-118,551 (erythro-(I)-I-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride), a selective β_2-adrenoceptor antagonist, into the knee joint at a concentration of 30 μg ml^{-1}. Although ICI-118,551 has not been examined for cross-binding with the nicotinic receptor, the specificity for this drug has been demonstrated by pharmacological (Chojnacka-Wojcik & Przegalinski, 1991; Veltmar *et al*., 1992) as well as receptor binding approaches (Bjornerheim *et al*., 1991; Pauwels *et al*., 1991). The dose we used in this experiment was taken from studies which showed that ICI-118,551 is effective in antagonizing the inhibition of BK-induced plasma extravasation by salbutamol (a selective β_2-adrenoceptor agonist) (Coderre *et al*., 1990; 1991). ICI-118,551 was injected intra-articularly so as to achieve minimum systemic action. To demonstrate this and to identify its site of action one knee was perfused with ICI-118,551 and BK and the contralateral knee, as a control, received BK alone.

Naloxone We administered naloxone, an opioid receptor antagonist, to explore the contribution of endogenous opioids to inhibition of plasma extravasation by s.c. nicotine. Naloxone was given 30 min before the beginning of the experiments (two hourly injections of 1 mg kg^{-1}, i.p.). This dose of naloxone has been used previously and shown to be effective in antagonizing the effects of opioid receptor agonists (Taguchi *et al*., 1993).

RU-38,486 (mifepristone) To examine the contribution of glucocorticoids to nicotine-induced inhibition of BK- or PAF-induced plasma extravasation, we pretreated some rats, 3 h before the commencement of the knee joint perfusion, with RU-38,486 (30 mg kg^{-1}, s.c.), a glucocorticoid and progesterone receptor antagonist. A similar protocol with RU-38,486 at this dose has been found to be effective in antagonizing actions mediated by glucocorticoid receptors in other systems (Peeters *et al*., 1992).

Metyrapone Since RU-38,486 inhibits both glucocorticoid and progesterone receptors (Philibert *et al*., 1989), we also studied groups of rats treated with metyrapone (two hourly injections of 100 mg kg^{-1}, i.p.), a substance which blocks glucocorticoid synthesis (MacNiven & de Catanzaro, 1990) with no inhibitory actions on progesterone-mediated effects (Rondinone *et al*., 1992). Metyrapone is believed to deplete glucocorticoids rapidly by inhibiting the conversion of 11-deoxy-corticosterone to corticosterone (Herman *et al*., 1992). This protocol of metyrapone administration has been found to be effective in inhibiting adrenal corticosteroid synthesis (Jacobson *et al*., 1989; Philibert *et al*., 1989; Herman *et al*., 1992; Green *et al*., 1995).

Mecamylamine To examine if spinal nicotinic receptors are activated by s.c. nicotine, we administered mecamylamine spinally. The dose of mecamylamine used in the present intrathecal protocol (0.025 mg kg^{-1}) has been found to be ineffective, when given i.v., in affecting s.c. nicotine inhibition of BK-induced plasma extravasation (Miao *et al*., 1994).

Mecamylamine was injected into the lumbar section of the spinal cord via a PE 10 catheter (i.d. 0.28 mm, o.d. 0.61 mm; Clay Adams, Division of Becton Dickinson and Company, Parsippany, NJ) chronically implanted by the method of Yaksh and Rudy (1977) 7 days before the experiments.

Since i.t. mecamylamine (equivalent to 0.167 mg kg^{-1}, a dose approximately 7 times higher than the one we used in the present study) reverses i.t. nicotine (equivalent to 1.42–1.67 mg kg^{-1})-induced hypertension (from normal blood pressure of 100–110 mmHg to 145–155 mmHg) (Khan *et al*., 1994), we conducted experiments to examine if i.t. mecamylamine 0.025 mg kg^{-1} affects systemic blood pressure. In order to conduct a paired comparison for the action of i.t. mecamylamine, BK-induced plasma extravasation and systemic blood pressure were simultaneously measured over 5 min intervals. First intrathecal normal saline was administered, after the level of BK-induced plasma extravasation and systemic blood pressure were stable. Mecamylamine (0.025 mg kg^{-1}) at the same volume (10 μl) was injected 15 min after i.t. normal saline (10 μl). In these experiments, we found that neither BK-induced plasma extravasation nor systemic blood pressure was affected by i.t. normal saline (BK-induced plasma extravasation: 0.106 ± 0.11 vs. 0.112 ± 0.14 absorbance at 620 nm, $n = 8$; systemic blood pressure: 94 ± 7 vs. 89 ± 5 mmHg, $n = 4$, both

$P>0.05$, Student's t test; representing 5.66% increase in plasma extravasation and 4.84% decrease in blood pressure) or by i.t. mecamylamine (BK-induced plasma extravasation: 0.106 ± 0.11 vs. 0.110 ± 0.14 absorbance at 620 nm, $n=8$; systemic blood pressure: 94 ± 7 vs. 92 ± 5 mmHg, $n=4$, both $P>0.05$; representing 3.77% increase in plasma extravasation and 2.61% decrease in blood pressure).

Combination of ICI-118,551, RU-38,486 and naloxone To examine if there is more than one mechanism which mediates the action of nicotine, we used combined administration of agents that act through the three proposed pathways. In this experiment, RU-38,486 was administered (30 mg kg^{-1}, s.c.) 3 h before the knee joint perfusion was started, naloxone (two hourly injections of 1 mg kg^{-1}, i.p.) was given 30 min before the start of the knee joint perfusion and ICI-118,551 (30 μg ml^{-1}) was co-perfused with BK (160 ng ml^{-1}) intra-articularly into the knee joint.

Surgical procedures

Adrenal medullectomy To assess the contribution of the adrenal medulla to the effects of nicotine on plasma extravasation we performed bilateral surgical adrenal medullectomy, as previously described (Wilkinson *et al*., 1981; Miao *et al*., 1993). Under ether anaesthesia, the adrenal gland was located through an incision in the lateral abdominal wall, an incision made in the adrenal gland and the adrenal medulla enucleated. Rats were given 0.5% saline, in place of water, to drink for the first 7 days after surgery (Wilkinson *et al*,. 1981). Since incising the adrenal gland causes trauma to the adrenal cortex, adrenal medullectomy was performed at least 5 weeks before the knee joint perfusion experiments to allow recovery of function of the adrenal cortex; but the stress-induced increase of plasma corticosterone is still somewhat lower than in normal rats at this time (Wilkinson *et al*., 1981). The magnitudes of the response of BK and PAF-induced plasma extravasation in the different experimental groups were, with some exceptions (see Table 1), not significantly different.

Statistics

Data are presented as mean $\pm$ s.e.mean. The plateau levels of BK- or PAF-induced plasma extravasation, before injection of nicotine, served as control values. The maximum decrease of BK- or PAF-induced plasma extravasation over a period of 45 min after injection of nicotine was recorded and converted into percentage change from the control plateau value obtained before administration of nicotine (i.e., magnitude of inhibition by nicotine) (Miao *et al*., 1992b). Significant differences between maximum inhibition for pairs of groups of rats, produced after s.c., injection of nicotine, were determined by unpaired Student's t test. Differences were considered statistically significant at a P value <0.05.

Materials

The drugs used in the present experiments were: bradykinin acetate, platelet activating factor (PAF, L-α-phosphatidylcholine, β-acetl-γ-o-hexadecyl), bovine serum albumin (BSA), mecamylamine hydrochloride, nicotine hydrogen tartrate, naloxone hydrochloride, dimethyl sulphoxide (DMSO), and peanut oil from Sigma Chemical Co., St. Louis, MO; ICI-118, 551 [erythro-($\pm$)-I(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride] from Cambridge Research Biochemicals Incorporated (Gadbrook Park Northwich, Cheshire, U.K.). RU-38,486 (mifepristone) and metyrapone (metopirone) were generous gifts from Roussel Uclaf, France and Ciba-Geigy Pharmaceutical Co., Ardsley, NY, respectively. PAF was dissolved in freshly prepared 0.25% BSA in normal saline solution. ICI-118,551 was initially dissolved in 100% ethanol in trace volume, and further diluted with normal saline (final concentration of ethanol $<0.05\%$). RU-38,486 was suspended in peanut oil. Metyrapone was dissolved in DMSO. The remaining compounds were dissolved in normal saline.

Results

Effects of nicotine (s.c.) on BK- and PAF-induced plasma extravasation

Nicotine (s.c.) inhibited BK- and PAF-induced plasma extravasation (Figures 1–4). The dose-response relationship produced by nicotine given by the cumulative dosing regimen was similar to that obtained from experiments with nicotine at low, mid, and high doses, based on the non-cumulative dosing regimen (data not shown). Results from non-cumulative dosing regimen

Table 1 The effects of surgical and pharmacological interventions on nicotine-induced inhibition of bradykinin- or platelet activating factor-induced plasma extravasation

Perfusion	*Intervention*	*Plateau PE before Nic*[a]	*Max inhibition by Nic 0.01 μg kg^{-1}*[b]	*Max inhibition by Nic 1 mg kg^{-1}*[b]
BK	Control	0.187 ± 0.01	25.6 ± 1.8	62.7 ± 3.8
BK	Adrenal medullectomy	0.175 ± 0.01	10.1 ± 1.7*	32.3 ± 3.8*
BK	ICI-118,551	0.343 ± 0.03*	26.6 ± 3.7	38.5 ± 6.0*
BK	Naloxone	0.231 ± 0.03	9.5 ± 6.3*	33.7 ± 3.5*
BK	RU-38,486	0.236 ± 0.02	27.9 ± 3.9	18.4 ± 1.5*
BK	Metyrapone	0.104 ± 0.08*	21.0 ± 5.0	25.4 ± 2.3*
BK	Mecamylamine	0.228 ± 0.03	29.9 ± 2.9	7.7 ± 8.4*
PAF	Control	0.305 ± 0.02	23.3 ± 5.1	49.5 ± 3.3
PAF	Adrenal medullectomy	0.278 ± 0.02	9.6 ± 2.3*	26.2 ± 2.6*
PAF	ICI-118,551	0.330 ± 0.03	31.7 ± 2.7	49.8 ± 2.7
PAF	Naloxone	0.321 ± 0.03	12.3 ± 4.7*	22.1 ± 2.5*
PAF	RU-38,486	0.359 ± 0.03*	30.9 ± 2.4	11.2 ± 3.1*
PAF	Metyrapone	0.181 ± 0.02*	29.6 ± 2.6	24.8 ± 2.7*
PAF	Mecamylamine	0.305 ± 0.03	30.6 ± 3.8	20.6 ± 5.2*

BK: bradykinin; PAF: platelet activating factor; Nic: nicotine; PE: plasma extravasation. [a]Plateau plasma extravasation measured by absorbance at 620 nm, a parameter which is linearly related to the concentration of the blue dye; for each preparation, $n=16$. [b]Maximum inhibition of plasma extravasation by s.c. nicotine measured as % of the control plateau level of plasma extravasation; for each preparation $n=8$. The significant differences from the normal control of BK- or PAF-induced plasma extravasation are shown as *$P<0.05$ (unpaired Student's t test).

experiments showed that 1 mg kg^{-1} nicotine produces inhibitory effects on BK-induced plasma extravasation approximately at the ED_{95} dose, estimated based on the cumulative dose-response curve; nicotine 3 μg kg^{-1} produced an effect approximately equal to the ED_{60} while 0.01 μg kg^{-1} produced an effect less than the estimated ED_{10}. Inhibitory action of single doses of nicotine on PAF-induced plasma extravasation was as follows: 1 mg kg^{-1} approximately ED_{70}; 3 and 0.01 μg kg^{-1} both approximately equal to the estimated ED_{25} (data not shown).

Effects of surgical and pharmacological interventions on actions of nicotine

Effect of adrenal medullectomy To examine the contribution of the adrenal medulla, we studied the effect of adrenal medullectomy on actions produced by nicotine. Inhibition of BK- or PAF-induced plasma extravasation by nicotine at low (0.01 μg kg^{-1}) and high (1 mg kg^{-1}) doses was significantly attenuated by bilateral adrenal medullectomy (all $P<0.01$) (Figure 1a and b and Table 1).

Effect of intra-articularly administered β_2-adrenoceptor antagonist To determine if adrenaline, an adrenal medullary β_2-adrenoceptor agonist released by nicotine, mediates the decrease of BK-induced plasma extravasation, we next examined the effect of blocking local β_2-adrenoceptors by intra-articular ICI-118,551 (30 μg ml^{-1}) on the actions of nicotine. The inhibitory action produced by high-dose nicotine on BK-induced plasma extravasation, was significantly attenuated by ICI-118,551 ($P<0.01$) (Figure 1a and Table 1). The effect of low-dose nicotine on BK-induced plasma extravasation and the inhibitory action of both doses of nicotine on PAF-induced plasma extravasation were not significantly affected by ICI-118,551 ($P>0.05$) (Figure 1a and b and Table 1).

To rule out a contribution of β_2-adrenoceptors at extra-articular sites, we conducted another experiment in which one knee was perfused with BK and ICI-118,551, while the contralateral knee, serving as a control, was perfused with BK alone. Since only the action produced by high-dose nicotine is mediated by β_2-adrenoceptors (Figure 1a), this particular dose was used to test the contribution of systemic action of adrenaline to the nicotine inhibition of BK-induced plasma extravasation. The inhibitory effect of nicotine on BK-induced plasma extravasation was significantly attenuated in knees which received intra-articular ICI-118,551 but was not affected in the contralateral knee ($P<0.01$) (data not shown).

Effect of systemically administered opioid receptor antagonist To evaluate the role of opioids in nicotine inhibition of plasma extravasation, we examined the effect of blocking opioid receptors by naloxone on the action of nicotine. Inhibition of BK- or PAF-induced plasma extravasation by nicotine at both doses was significantly attenuated by systemic naloxone (two hourly injections of 1 mg kg^{-1}, i.p.) ($P<0.01$) (Figure 1a and b and Table 1).

Effects of administration of inhibitors of glucocorticoids Since nicotine also stimulates release of glucocorticoids, a class of potent anti-inflammatory agents, we examined a possible contribution of endogenous glucocorticoids to the action of nicotine. High-dose nicotines inhibition of BK- or PAF-induced plasma extravasation was significantly attenuated by RU-38,486 (30 mg kg^{-1}, s.c.) or by metyrapone (two hourly injections of 100 mg kg^{-1}, i.p.) (all $P<0.01$) (Figure 2a and b and Table 1). The actions of low-dose nicotine were not affected by these treatments (all $P>0.05$) (Figure 2a and b and Table 1).

Effects of intrathecal mecamylamine Since nicotine administered s.c. may cross the blood-brain barrier and nicotinic receptors in the spinal cord may be involved in the activation of the hypothalamo-pituitary-adrenal axis (Krueger *et al*., 1991), we also examined effects of blocking the spinal nicotinic receptors on the action of individual doses of nicotine. Inhibition of BK- or PAF-induced plasma extravasation produced by high-dose, but not low-dose, nicotine was significantly attenuated by i.t. mecamylamine (0.025 mg kg^{-1}) (both $P<0.01$) (Figure 3a and b and Table 1).

Effects of the combination of ICI-118,551, RU-38,486 and naloxone Since the inhibitory action of nicotine may be mediated by one common mechanism or by multiple independent mechanisms, we examined the effects of the combined administration of agents that act through the three proposed pathways, on high- and low-dose nicotine inhibition of BK-induced plasma extravasation. In the combined treatment group, we used RU-

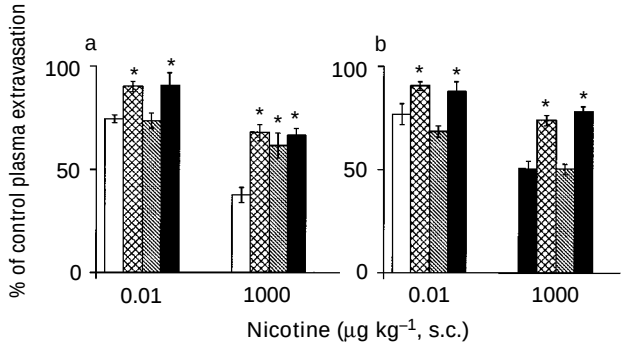

Figure 1 Effect of adrenal medullectomy (cross-hatched columns), ICI-118,551 (30 μg ml^{-1}, intra-articularly) (hatched columns) and naloxone (two hourly injections of 1 mg kg^{-1}, i.p.) (solid columns) on nicotine-induced inhibition of (a) BK- and (b) PAF-induced plasma extravasation in the knee joint of the rat. (a) Nicotine inhibited BK-induced plasma extravasation in normal rats (open columns, $n=8$). In adrenal medullectomized rats, nicotine inhibition of BK-induced was attenuated (cross-hatched columns, $n=8$). ICI-118,551 did not affect actions produced by low-dose nicotine but attenuated that of high-dose nicotine (hatched columns, $n=8$). Naloxone attenuated the actions of nicotine (solid columns, $n=8$). (b) Nicotine also inhibited PAF-induced plasma extravasation in normal rats (open columns, $n=8$), which was attenuated by adrenal medullectomy (cross-hatched columns, $n=8$) but not affected by ICI-118,551 (hatched columns, $n=8$). Naloxone attenuated the actions of nicotine (solid columns, $n=8$). *Significantly different from the action produced by the same dose of nicotine in control rats.

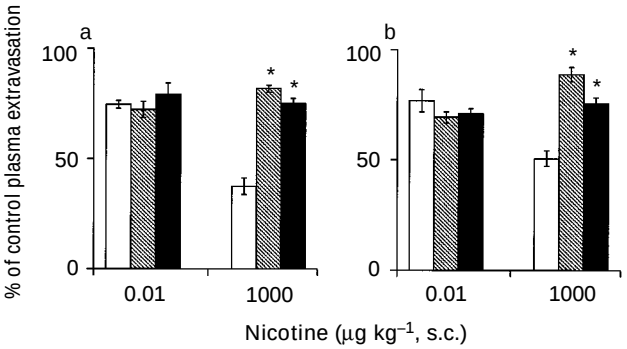

Figure 2 Effects of RU-38,486 (30 mg kg^{-1}, s.c.) (hatched columns) and metyrapone (two hourly injections of 100 mg kg^{-1}, i.p.) (solid columns) on action of nicotine in inhibiting (a) BK- and (b) PAF-induced plasma extravasation. Open columns represent nicotine-induced inhibition of plasma extravasation in normal rats. (a) RU-38,486 attenuated high-dose nicotine inhibition of BK-induced plasma extravasation without affecting that of low-dose nicotine (hatched columns, $n=8$). Metyrapone also attenuated the action of high-dose, but not low-dose, nicotine (solid columns, $n=8$). (b) RU-38,486 attenuated the inhibitory action of nicotine on PAF-induced plasma extravasation at high-dose without affecting that of low-dose nicotine (hatched columns, $n=8$). Metyrapone also had a similar action (solid columns, $n=8$).

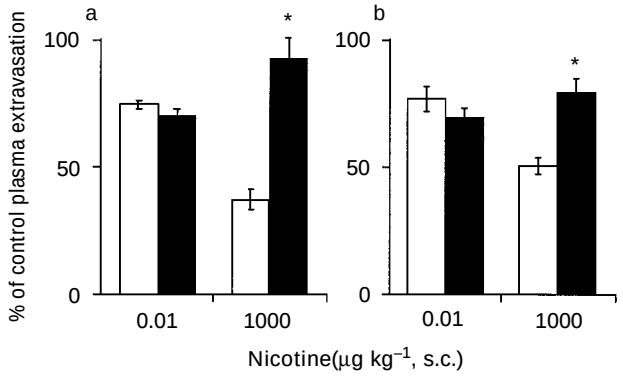

Figure 3 Effects of intrathecal (i.t.) mecamylamine (0.025 mg kg^{-1}) (solid columns) on nicotine inhibition of (a) BK- and (b) PAF-induced plasma extravasation. Open columns represent nicotine-induced inhibition of plasma extravasation in normal rats. Mecamylamine attenuated high-dose nicotine inhibition of BK-induced plasma extravasation without affecting actions produced by low-dose nicotine ($n=8$). (b) Mecamylamine also attenuated the inhibitory action of high-dose, but not low-dose, nicotine on PAF-induced plasma extravasation ($n=8$).

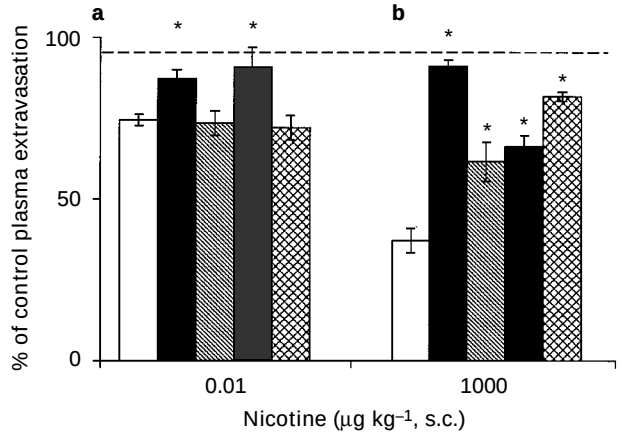

Figure 4 Effects of combined treatment with RU-38,486 (30 mg kg^{-1}, s.c.), naloxone (two hourly injections of 1 mg kg^{-1}, i.p.) and ICI-118,551 (30 μg ml^{-1}, intra-articularly) on low- (a) and high-dose (b) nicotine inhibition of BK-induced plasma extravasation. (a) Combined treatment attenuated low-dose nicotine inhibition of BK-induced plasma extravasation (solid column, $n=8$) by an amount similar to that of naloxone alone (stippled column) ($P>0.05$) but greater than that of control (without treatment) (open column, $n=8$), ICI-118,551 alone (hatched column, $n=8$), or RU-38,486 alone (cross-hatched column, $n=8$) (all $P<0.05$). (b) Combined treatment attenuated high-dose nicotine inhibition of BK-induced plasma extravasation (solid column, $n=8$) by an amount significantly greater than any of the antagonists alone (naloxone alone: stippled column, $n=8$; ICI-118,551 alone; hatched column, $n=8$; RU-38,486 alone: cross-hatched column, $n=8$) or the control (open column, $n=8$) (all $P<0.01$). Dotted line represents changes of BK-induced plasma extravasation in rats that received nicotine-free vehicle injection over the same period of time as that after nicotine injection. *Significantly different from the action produced by the same dose of nicotine in the controls (i.e., without treatment).

38,486 (30 mg kg^{-1}, s.c.), naloxone (two hourly injections of 1 mg kg^{-1}, i.p.) and ICI-118,551 (30 μg ml^{-1}, intra-articularly) to block the pathways mediated by glucocorticoids, opioids and adrenaline, respectively. Compared with the effect of individual antagonists, the attenuation of low-dose nicotine inhibition was not significantly different from that of the combined treatment ($P>0.05$) (Figure 4). However, the attenuation of high-dose nicotine was significantly greater in the combined treatment group when it was compared with the effects of individual antagonist ($P<0.05$) (Figure 4).

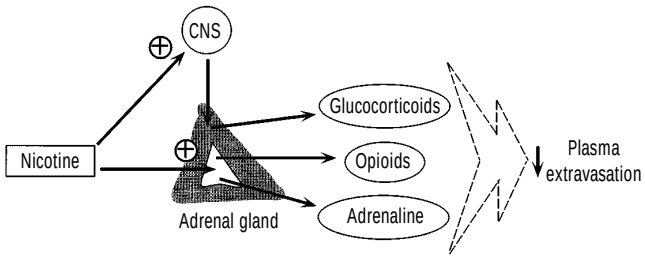

Figure 5 Schematic diagram of proposed mechanism(s) underlying the inhibitory action of s.c., nicotine on synovial plasma extravasation. Nicotine is known to excite the adrenal medulla to release adrenaline (Cryer *et al*., 1976) and opioids (Wilson, 1991); and, via indirect actions, to release glucocorticoids (Krueger *et al*., 1991). These adrenal hormones contribute to the decrease in plasma extravasation produced by nicotine. In addition, part of the effect of high-dose nicotine is mediated by spinal nicotinic mechanisms.

Discussion

In this study we confirm our previous finding that s.c. nicotine inhibits BK-induced synovial plasma extravasation (Miao *et al*., 1992a) and extend these studies to determine the underlying mechanisms. We found that nicotine inhibits BK- or PAF-induced plasma extravasation, which was partially attenuated by adrenal medullectomy. However, ICI-118,551, an antagonist blocking the major receptors for adrenaline (a key adrenal medullary hormone), only attenuated the inhibitory actions of high-dose nicotine on BK-, but not PAF-, induced plasma extravasation without affecting those of low-dose nicotine. Therefore, adrenaline is unlikely to mediate all the adrenal medulla-dependent actions of nicotine. Since the adrenal medulla also releases opioids upon stimulation by nicotine (Wilson, 1991), which can block synovial plasma extravasation as well (Green & Levine, 1992), we used an opioid receptor antagonist to examine their contribution to the inhibitory action of nicotine. Our findings suggest that opioids contribute to the inhibitory effects of all doses of nicotine. The contribution of neuropeptide Y (NPY), another adrenal medullary hormone released by nicotine (Hexum & Rusett, 1989) that can block synovial plasma extravasation (Green *et al*., 1993b), was not examined because of the lack of an effective receptor antagonist.

The observation that ICI-118,551 treatment in one knee did not affect the action of nicotine in the contralateral knee suggests that local β_2-adrenoceptors in the joint mediate the inhibitory action of nicotine. The β_2-adrenoceptor-mediated effect of nicotine affected only plasma extravasation induced by BK, which is dependent on sympathetic postganglionic neurone terminals in the joint at the dose used in the present study (Coderre *et al*., 1989; Miao *et al*,. 1996a,b), but not that induced by PAF, an inflammatory mediator which is independent of nerve innervation (Green *et al*., 1993a). This finding further indicates that the sympathetic postganglionic neurone terminal in the joint is the site at which β_2-adrenoceptor agonists act to inhibit plasma extravasation.

In addition to adrenal medullary hormones, we also explored the role of adrenal cortical hormones in mediating the inhibitory action of nicotine because they can be released by nicotine (Pomerleau & Pomerleau, 1990; Donnerer & Lembeck, 1990; Krueger *et al*., 1991) and inhibit plasma extravasation in extra-articular tissues (Tsurufuji *et al*., 1980; Seghaye *et al*., 1986; Brattsand *et al*., 1991). At a dose of 1 mg kg^{-1} (a dose similar to those previously found to release glucocorticoids, i.e., 0.25 mg kg^{-1} in Donnerer & Lembeck, 1990 and 4.5 mg kg^{-1} in Krueger *et al*., 1991), nicotine inhibition of BK- and PAF-induced plasma extravasation was attenuated by the glucocorticoid receptor antagonist (RU-38,486) or by the glucocorticoid synthesis inhibitor (metyr-

apone). These observations support the notion that glucocorticoids also mediate the action of nicotine. While it was not possible to evaluate the intra-articular effect of RU-38,486 (i.e., its vehicle for administration is peanut oil), given its well-documented efficacy when administered locally into inflamed tissue, we suggest a local site of action of glucocorticoids in the joint.

The finding that the inhibitory action of a high-dose nicotine was attenuated by i.t. mecamylamine implicates the involvement of a spinal nicotinic mechanism. While the exact mechanism is not yet clear, possible mechanisms include a spinal ascending pathway (Miao *et al*., 1994) that projects to the hypothalamus and excites the hypothalamo-pituitary adrenal axis to release glucocorticoids and a spinal circuit which reduces regional blood flow by enhancing sympathetic outflow, that would non-selectively decrease plasma extravasation.

In an attempt to understand if these mechanisms of action (i.e., pathways mediated by adrenaline, opioids and glucocorticoids) are independent ones, we examined the effect of combined treatment by blocking receptors of these adrenal hormones on the magnitude of attenuated action of nicotine. We found that although the action of low-dose nicotine in the combined treatment group was not different from that produced by naloxone alone (note ICI-118,551 or RU-38,486 alone did not affect low-dose nicotine action), the action of high-dose nicotine in the combined treatment group was significantly less than that produced by any of the single antagonists. Therefore, we believe that the neural and endocrine systems mediated, respectively, by adrenaline, opioids and glucocorticoids are, in fact, independent mechanisms. A schematic illustration of the proposed mechanisms is presented in Figure 5.

In this study, nicotine, a selective agonist activating nicotinic cholinoceptors, was used as a tool to assess cholinergic control of neural and endocrine systems which may play a role in regulating microvascular permeability. An investigation into the role of nicotinic cholinergic circuitry in the regulation of the inflammatory response, including pain and vascular changes, will be important to the understanding of the physiological mechanisms which regulate the inflammatory process. Results from our study provide evidence to suggest that several adrenal hormones, which can be released by nicotine, mediate inhibition of synovial plasma extravasation.

This work was supported by a grant from the Council for Tobacco Research. The authors wish to thank Dr Mary F. Dallman for many helpful discussions and a critical review of the manuscript.

References

APPLEYARD, C.B. & HILLER, K. (1995). Biosynthesis of platelet-activating factor in normal and inflamed human colon mucosa: evidence for the involvement of the pathway of platelet-activating factor synthesis de novo in inflammatory bowel disease. *Clin. Sci*., **88,** 713 – 717.

BJORNERHEIM, R., GOLF, S. & HANSSON, V. (1991). Specific non-beta-adrenergic binding sites for ^{125}I-iodocyanopindolol in myocardial membrane preparations: a comparative study between human, rat and porcine hearts. *Cardiovasc. Res*., **25,** 764 – 773.

BRATTSAND, R., O'DONNELL, S.R., MILLER-LARSSON, A. & RAUCHLE, K.L. (1991). Attenuation of bradykinin-induced mucosal inflammation by topical budesonide in rat trachea. *Agents Actions*, **34,** 200 – 202.

CARR, J. & WILHELM, D.L. (1964). The evaluation of increased vascular permeability in the skin of guinea pigs. *Aust. J. Exp. Biol. Med. Sci*., **42,** 511 – 522.

CHOJNACKA-WOJCIK, E. & PRZEGALINSKI, E. (1991). Evidence for the involvement of 5-HT1A receptors in the anticonflict effect of ipsapirone in rats. *Neuropharmacology*, **30,** 703 – 709.

CODERRE, T.J., BASBAUM, A.I., DALLMAN, M.F., HELMS, C. & LEVINE, J.D. (1990). Epinephrine exacerbates arthritis by an action at presynaptic beta2-adrenoceptors. *Neuroscience*, **34,** 521 – 523.

CODERRE, T.J., BASBAUM, A.I. & LEVINE, J.D. (1989). Neural control of vascular permeability: Interactions between primary afferents, mast cells, and sympathetic efferents. *J. Neurophysiol*., **62,** 48 – 58.

CODERRE, T.J., CHAN, A.K., HELMS, C., BASBAUM, A.I. & LEVINE, J.D. (1991). Increasing sympathetic nerve terminal-dependent plasma extravasation correlates with decreased arthritic joint injury in rats. *Neuroscience*, **40,** 185 – 189.

CRYER, P.E., HAYMOND, M.W., SANTIAGO, J.V. & SHAH, S.D. (1976). Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *New Engl. J. Med*., **295,** 573 – 577.

DONNERER, J. & LEMBECK, F. (1990). Different control of the adrenocorticotropin-corticosterone response and of prolactin secretion during cold stress, anesthesia, surgery, and nicotine injection in the rat: involvement of capsaicin-sensitive sensory neurons. *Endocrinology*, **126,** 921 – 926.

GREEN, P.G. & LEVINE, J.D. (1992). δ- and κ-opioid agonists inhibit plasma extravasation induced by bradykinin in the knee joint of the rat. *Neuroscience*, **49,** 129 – 133.

GREEN, P.G., LUO, J., HELLER, P.H. & LEVINE, J.D. (1993a). Neurogenic and non-neurogenic mechanisms of plasma extravasation in the rat. *Neuroscience*, **52,** 735 – 743.

GREEN, P.G., LUO, J., HELLER, P.H. & LEVINE, J.D. (1993b). Modulation of bradykinin-induced plasma extravasation in the rat knee joint by sympathetic co-transmitters. *Neuroscience*, **52,** 451 – 458.

GREEN, P.G., MIAO, F.J.-P., JÄNIG, W. & LEVINE, J.D. (1995). Negative feedback neuroendocrine control of the inflammatory response in rats. *J. Neurosci*., **15,** 4678 – 4686.

HARGREAVES, K.M., ROSZKOWSKI, M.T. & SWIFT, J.Q. (1993). Bradykinin and inflammatory pain. *Agents Actions*., **41,** (Suppl), 65 – 73.

HERMAN, J.P., SCHAFER, M.K., THOMPSON, R.C. & WATSON, S.J. (1992). Rapid regulation of corticotropin-releasing hormone gene transcription in vivo. *Mol. Endocrinol*., **6,** 1061 – 1069.

HEXUM, T.D. & RUSETT, L.R. (1989). Stimulation of cholinergic receptor mediated secretion from the bovine adrenal medulla by neuropeptide Y. *Neuropeptides*, **13,** 35 – 41.

JACOBSON, L., AKANA, S.F., CASCIO, C.S., SCRIBNER, K., SHINSAKO, J. & DALLMAN, M.F. (1989). The adrenocortical system responds slowly to removal of corticosterone in the absence of concurrent stress. *Endocrinology*, **124,** 2144 – 2152.

KHAN, I.M., TAYLOR, P. & YAKSH, T.L. (1994). Stimulatory pathways and sites of action of intrathecally administered nicotinic agents. *J. Pharmacol. Exp. Ther*., **271,** 1550 – 1557.

KRUEGER, R.J., LIN, C.M. & FUNG, Y.K. (1991). Effect of chronic administration of nicotine or cocaine on steroidogenesis in rat adrenocortical cells. *J. Pharmac. Pharmacol*., **43,** 200 – 203.

MACNIVEN, E. & DE CATANZARO, D. (1990). Reversal of stress-induced pregnancy blocks in mice by progesterone and metyrapone. *Physiol. Behav*., **47,** 443 – 448.

MATTA, S.G., FOSTER, C.A. & SHARP, B.M. (1993). Nicotine stimulates the expression of cFos protein in the parvocellular paraventricular nucleus and brainstem catecholaminergic regions. *Endocrinology*, **132,** 2149 – 2156.

MATTA, S.G., MCALLEN, K.M. & SHARP, B.M. (1990). Role of the fourth cerebroventricle in mediating rat plasma ACTH responses to intravenous nicotine. *J. Pharmacol. Exp. Ther*., **252,** 623 – 630.

MCKAY, D.B. & TRENT-SANCHEZ, P. (1990). Effect of noncompetitive nicotinic receptor blockers on catecholamine release from cultured adrenal chromaffin cells. *Pharmacology*, **40,** 224 – 230.

MIAO, F.J.-P., BENOWITZ, N.L., BASBAUM, A.I. & LEVINE, J.D. (1992a). Sympathoadrenal contribution to nicotine and muscarinic modulation of bradykinin-induced plasma extravasation in the knee joint of the rat. *J. Pharmacol. Exp. Ther*., **262,** 889 – 895.

MIAO, F.J.-P., DALLMAN, M.F., BENOWITZ, N.L., BASBAUM, A.I. & LEVINE, J.D. (1993). Adrenal medullary modulation of the inhibition of bradykinin-induced plasma extravasation. *J. Pharmacol. Exp. Ther*., **264,** 839 – 844.

MIAO, F.J.-P., GREEN, P., CODERRE, T.J., JÄNIG, W. & LEVINE, J.D. (1996a) Sympathetic-dependent and -independent mechanisms for synovial plasma extravasation induced by bradykinin is dose-dependent. *Neurosci. Lett.*, **205,** 165–168.

MIAO, F.J.-P., HELMS, C., BENOWITZ, N.L., BASBAUM, A.I. & LEVINE, J.D. (1992b). Chronically-administered nicotine attenuates bradykinin-induced plasma extravasation and aggravates arthritis-induced joint injury in the rat. *Neuroscience*, **51,** 649–655.

MIAO, F.J.-P., JÄNIG, W., DALLMAN, M.F., BENOWITZ, N.L., HELLER, P.H., BASBAUM, A.I. & LEVINE, J.D. (1994). Role of vagal afferents and spinal pathways modulating bradykinin-induced plasma extravasation by intrathecal nicotine. *J. Neurophysiol.*, **72,** 1199–1207.

MIAO, F.J.-P., JÄNIG, W. & LEVINE, J.D. (1996b). Contribution of sympathetic postganglionic nerve terminals to bradykinin-induced synovial plasma extravasation. *J. Neurophysiol.*, **75,** 715–724.

PAUWELS, R.J., VAN GOMPEL, P. & LEYSEN, J.E. (1991). Human beta 1- and beta 2-adrenergic receptor binding and mediated accumulation of cAMP in transfected Chinese hamster ovary cells. Profile of nebivolol and known beta-adrenergic blockers. *Biochem. Pharmacol.*, **42,** 1683–1689.

PEETERS, B.W., SMETS, R.J. & BROEKKAMP, C.L. (1992). The involvement of glucocorticoids in the acquired immobility response is dependent on the water temperature. *Physiol. Behav.*, **51,** 127–129.

PHILIBERT, D., HARDY, M., GAILLARD-MOGUILEWSKY, M., NIQUE, F., TOURNEMINE, C. & NEDELEC, L. (1989). New analogues of mifepristone with more dissociated antiprogresterone activities. *J. Steroid Biochem.*, **34,** 413–417.

POMERLEAU, O.F. & POMERLEAU, C.S. (1990). Cortisol response to a psychological stressor and/or nicotine. *Pharmacol. Biochem. Behav.*, **36,** 211–213.

ROGERS, D.T. & IWAMOTO, E.T. (1993). Multiple spinal mediators in parenteral nicotine-induced antinociception. *J. Pharmacol. Exp. Ther.*, **267,** 341–349.

RONDINONE, C.M., BAKER, M.E. & RODBARD, D. (1992). Progestins stimulate the differentiation of 3T3-L1 preadipocytes. *J. Steroid Biochem. Mol. Biol.*, **42,** 795–802.

SEGHAYE, R., DEFLANDRE, E., REMACLE-VOLON, G. & DAMAS, J. (1986). Anti-inflammatory effect of ACTH in the rat. *Comptes Rendus Seances Soc. Biol. Sesfiliales*, **180,** 379–385.

STACHOWIAK, M.K., HONG, J.S. & VIVEROS, O.H. (1990). Co-ordinate and differential regulation of phenylethanolamine N-methyltransferase, tyrosine hydroxylase and proenkephalin mRNAs by neural and hormonal mechanisms in cultured bovine adrenal medullary cells. *Brain Res.*, **510,** 277–288.

STALKE, J., HADER, O., BAHR, V., HENSEN, J., SCHERER, G. & OELKERS, W. (1992). The role of vasopressin in the nicotine-induced stimulation of ACTH and cortisol in men. *Clin. Invest.*, **70,** 218–223.

STEEN, K.H., STEEN, A.E. & REEH, P.W. (1995). A dominant role of acid pH in inflammatory excitation and sensitization of nociceptors in rat skin, in vitro. *J. Neurosci.*, **15,** 3982–3989.

STEEN, K.H. & REEH, P.W. (1993). Actions of cholinergic agonists and antagonists on sensory nerve endings in rat skin, in vitro. *J. Neurophysiol.*, **70,** 397–405.

SWIFT, J.Q., GARRY, M.G., ROSZKOWSKI, M.T. & HARGREAVES, K.M. (1993). Effect of flurbiprofen on tissue levels of immunoreactive bradykinin and acute postoperative pain. *J. Oral. Max. Surg.*, **51,** 112–117.

TAGUCHI, K., HAGIWARA, Y., SUZUKI, Y. & KUBO, T. (1993). Effects of morphine on release of acetylcholine in the rat striatum: An in vivo microdialysis study. *Naunyn-Schiedeberg's Arch. Pharmacol.*, **347,** 9–13.

TSURUFUJI, S., SUGIO, K., TAKEMASA, F. & YOSHIZAWA, S. (1980). Blockade by antiglucocorticoids, actinomycin D and cycloheximide of anti-inflammatory action of dexamethasone against bradykinin. *J. Pharmacol. Exp. Ther.*, **212,** 225–231.

VELTMAR, A., CULMAN, J., QADRI, F., RASCHER, W. & UNGER, T. (1992). Involvement of adrenergic and angiotensinergic receptors in the paraventricular nucleus in the angiotensin II-induced vasopressin release. *J. Pharmacol. Exp. Ther.*, **263,** 1253–1260.

WILKINSON, C.W., SHINSAKO, J. & DALLMAN, M.F. (1981). Return of pituitary-adrenal function after adrenal enucleation or transplantation: diurnal rhythms and responses to ether. *Endocrinology*, **109,** 162–169.

WILL, P.C., THOMAS, T.K., IVERSON, L., BUCKMAN, D., WEIS, W., WILSON, C. & SRIVASTAVA, A. (1991). Platelet activating factor as a proinflammatory mediator in acetic-induced colitis in the rat. *Agents Actions*, **34,** 181–184.

WILSON, S.P. (1991). Regulation of proenkephalin synthesis in adrenal medullary chromaffin cells. *J. Neurochem.*, **56,** 945–952.

YAKSH, T.L. & RUDY, T.A. (1977). Studies on the direct spinal action of narcotics in the production of analgesia in the rat. *J. Pharmacol. Exp. Ther.*, **202,** 411–428.

(Received May 21, 1996
Revised September 16, 1996
Accepted October 2, 1996)