Br. J. Pharmac. (1980), **71**, 623–629

SUBSTANCE P INCREASES HYPOTHALAMIC BLOOD FLOW VIA AN INDIRECT ADRENERGIC-CHOLINERGIC INTERACTION

K.P. KLUGMAN, *F. LEMBECK, S. MARKOWITZ, G. MITCHELL & C. ROSENDORFF

MRC/University Circulation Research Unit, Department of Physiology, University of the Witwatersrand Medical School, Hospital Street, Johannesburg, South Africa and *University Institute for Experimental and Clinical Pharmacology, University of Graz, Universitatsplatz 4, A-8010 Graz, Austria

1 Hypothalamic blood flow (HBF) was measured in conscious rabbits by the ^{133}xenon washout technique.

2 Substance P in a dose of 50 or 500 ng increases HBF while 5 ng is without effect.

3 Cholinoceptor blockade, with either atropine or mecamylamine abolishes the vasodilator effect of substance P.

4 Chemical sympathectomy of the hypothalamus with 6-hydroxydopamine, or adrenoceptor blockade with either propranolol or phenoxybenzamine abolishes the effect of substance P on HBF.

5 Destruction of the intracerebral noradrenergic pathway (INP), or blockade of its vasodilator action, with barbiturate or bicarbonate, likewise prevent the vasodilator action of substance P.

6 These results suggest that substance P may cause an increase in HBF via the release of endogenous acetylcholine, which in turn stimulates the INP.

Introduction

Substance P is a potent dilator of many vascular beds (von Euler & Gaddum, 1931; Lofstrom, Pernow & Wahren, 1965; Maxwell, 1968; Hallberg & Pernow 1975; Eklund, Jogestrand & Pernow, 1977; Losay, Mroz, Tregear, Leeman & Gamble, 1977; Lembeck & Holzer, 1979). However, despite the high concentrations of substance P found in the brain and in the hypothalamus in particular (Mroz, Brownstein & Leeman, 1977), no data are available on the effects of substance P on cerebral blood flow. We have thus studied the effects of a variety of doses of synthetic substance P on hypothalamic blood flow (HBF) in the conscious rabbit.

It has been shown that an interaction occurs between the exogenous cholinomimetic, methacholine, and adrenergic fibres in the control of hypothalamic blood flow (Klugman, Mitchell & Rosendorff, 1979). More recent evidence indicates that endogenous acetylcholine can act on the intracerebral noradrenergic pathway (INP) to cause the release of noradrenaline and a subsequent increase in hypothalamic blood flow (Klugman, Mitchell & Rosendorff, 1980). Substance P has been postulated to cause the release of acetylcholine at certain autonomic neuroeffector junctions (Hedqvist & von Euler, 1977) as well as to activate cholinergic neurones innervating the ileal longitudinal muscle (Holzer & Lembeck, 1980).

In the central nervous system, an injection of substance P increases the turnover of acetylcholine (Malthe-Sorenssen, Chenex & Costa, 1978). In addition, substance P is found in the same fraction of synaptic vesicle and nerve endings as is acetylcholine (Kataska, 1962; Ryall, 1964). The possibility that the vascular effect of substance P in the hypothalamus may be indirect and contingent on the release of acetylcholine is investigated in this paper.

Methods

Twenty-nine New Zealand white rabbits (either sex) weighing between 2 and 4 kg were used in this study. The hypothalamus was chosen as the test region because it is a homogeneously perfused and relatively large region of grey matter, with discrete and quantitatively similar vascular supply to each side (Cranston & Rosendorff, 1971; Cameron & Caronna, 1976). In addition, it has a high concentration of substance P (Mroz *et al.*, 1977) and a high rate of turnover of acetylcholine (Phillis, 1970).

The technique used was a modification (Cranston & Rosendorff, 1971) of the method of Monnier & Gangloff (1961). Rabbits were anaesthetized with pentobarbitone sodium, 30 mg/kg (Abbott) and perspex

0007-1188/80/140615-07 $01.00

head-plates were screwed to their skulls. Holes drilled through the head-plates and the skull at co-ordinates aB on both sides of the midline allowed stereotaxic access to the anterior hypothalamus. All of the sites of injection were within 1 mm of the stereotaxic co-ordinate aB-16 mm using this technique (Rosendorff, 1972). Two weeks after fixation of the head-plates, hypothalamic blood flow (HBF) was measured in conscious rabbits by the ^{133}xenon (Amersham) clearance technique (Cranston & Rosendorff, 1971).

One side of the hypothalamus was designated the control side and the other, the test side; as the blood flow is equal on both sides of the hypothalamus (Cranston & Rosendorff, 1971; Cameron & Caronna, 1976), each rabbit could act as its own control. This aspect of the experimental design implies that similar systemic and local conditions exist at any moment for each half of the hypothalamus and any changes in HBF measured must be an effect of the locally injected substance.

Arterial blood pressure, blood gas tension and pH were measured every 10 min during the experiment. Blood pressure was measured via an indwelling catheter in the ear central artery and recorded on a Beckman dynograph with a Statham P23AA transducer and a strain gauge coupler. Blood samples for blood gas analysis and pH were taken from the ear artery catheter and measured on a blood gas analyzer (Instrumentation Laboratory 313)

At the time of the experiments, injection cannulae were so placed that their tips lay in identical positions in the hypothalamus on each side of the midline. Injections into the control side were 15 μCi ^{133}xenon dissolved in 5 μl phosphate buffered saline, pH 7.4. The test side received the ^{133}xenon in phosphate buffered saline plus the test substance. Injections were given on each side alternately at intervals of 10 min to allow the radioactivity to return to baseline levels.

After each injection the clearance of the radioactive isotope was measured for 500 s with an external collimated scintillation counter and recorded on magnetic tape (Hewlett-Packard). HBF was then calculated from the ^{133}xenon clearance curve on an IBM 370 computer using a non-linear regression analysis programme developed in our laboratory. HBF (ml 100 g^{-1} tissue min^{-1}) was obtained from the formula $HBF = \lambda B$, where B is the decay parameter of the monoexponential clearance curve and λ the tissue-blood partition coefficient for xenon. For the rabbit hypothalamus $\lambda = 0.74$ (Rosendorff & Luff, 1970). The changes in flow produced in each experiment were calculated by subtracting each test flow from the mean of the preceding and subsequent control flows. Each rabbit was used once only for each experiment and was used not more than three times in total. In each experiment, equal numbers of trials were performed on each animal to avoid individual bias.

The first series of experiments was designed to show the effect of synthetic substance P (Peninsula Laboratories, Inc.) on HBF. Doses ranging from 5 ng to 500 ng per 5 μl injection were used. Substance P was dissolved in 0.1 ml of 0.1 M acetic acid and then made up in phosphate buffered saline (pH 7.4) to the required dilution. Fresh solutions were made up each day.

Any effect of substance P may be a result of an effect of one of its constituent amino-acids. The effect on HBF of a mixture comprising the eleven amino-acids in the same ratio found in substance P was therefore also assessed.

The second part of the experiment was designed to elucidate the mode of action of substance P in changing HBF. Cholinoceptor blockade was achieved with atropine (M.L. Laboratories) and mecamylamine hydrochloride (Sigma).

Adrenoceptor blockade was obtained by use of phenoxybenzamine (S.K. and F.) and propranolol (I.C.I.). Adrenergic nerves in the hypothalamus and those running in the INP were destroyed in some animals by injection of 300 μg 6-hydroxydopamine (6-OHDA) (Sigma) into the hypothalamus, or brain stem reticular formation at co-ordinates aF-17 mm, respectively. (Monnier & Gangloff, 1961). Injections were made 4 days before the experiment. Although no histology was carried out to confirm the extent of the lesions, a lesion caused by 300 μg 6-OHDA effectively denervates one half of the rabbit hypothalamus or the INP in the reticular formation (Ungerstedt, 1971; Mitchell, Mitchell & Rosendorff, 1978) as both of these structures are of similar dimensions (Monnier & Gangloff, 1961).

Barbiturate (pentobarbitone sodium, Sigma) and bicarbonate (Merck) were used to characterize further the mode of action of substance P.

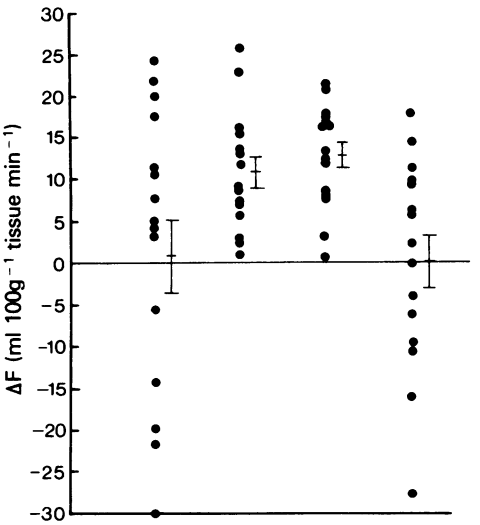
Tests of significance were based on the flow differences between the control and test sides, calculated from the several observations obtained on each animal for each procedure (Student's t test).

Results

In all rabbits tested there were no significant changes in blood pressure, blood gases or pH during the experiments.

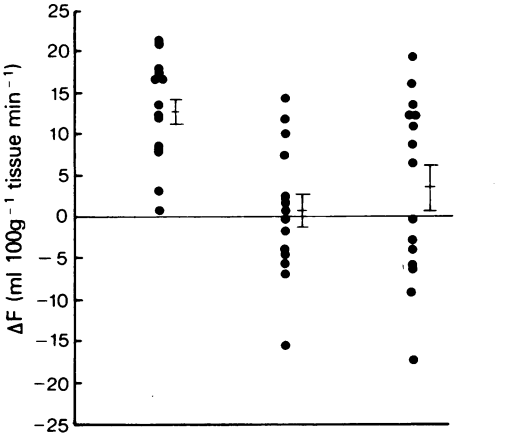
The effect of substance P on hypothalamic blood flow

The first part of the investigation established a dose-response curve for substance P (Figure 1). Intrahypothalamic injections of 50 and 500 ng substance P increase HBF significantly ($P < 0.001$). A dose of 5 ng per injection had no effect on HBF. Figure 1 also



ng	5 SP	50 SP	500 SP	500 SP mixture
	i.h.	i.h.	i.h.	i.h.
No. of Trials	15	15	15	15
x	0.7	10.6	12.7	0.0
s.e. mean	4.4	1.9	1.6	3.2
P	NS	<0.001	<0.001	NS

Figure 1 The effect of a mixture of the amino acids contained in substance P (SP) and of various doses of substance P on hypothalamic blood flow. Neither the amino acid mixture nor 5 ng substance P caused any significant change in hypothalamic flow; 50 and 500 ng substance P both caused significant increases in blood flow. i.h. = intra-hypothalamic injection. Δ F is the difference between the control and test flows, calculated as described in the method. *P* represents the significance of the deviation of Δ F from zero (Student's *t* test).



ng	500 SP	500 SP + 0.65μg Atropine	500 SP + 5μg Mecamylamine
	i.h.	i.h.	i.h.
No. of Trials	15	15	15
x	12.7	0.7	3.5
s.e. mean	1.6	2.0	2.8
P	<0.001	NS	NS

Figure 2 The effect of cholinoceptor blockade on Substance P (SP)-induced hypothalamic vasodilatation. Note that both atropine and mecamylamine abolish the vasodilatation due to 500 ng substance P.

shows that 500 ng of the mixture of substance P amino acids was without effect on HBF.

The effect of cholinoceptor blockade on the substance P-induced vasodilatation

Figure 2 shows the effect of cholinoceptor blockade on a vasodilator dose (500 ng) of substance P; 0.65 μg of the muscarinic antagonist, atropine, and 5 μg of the nicotinic antagonist, mecamylamine, both abolished the vasodilatation.

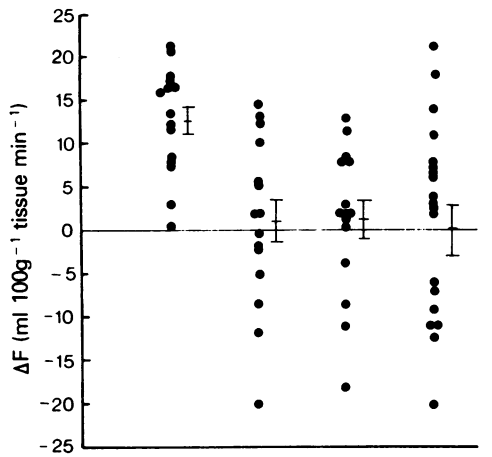
The effect of adrenoceptor blockade on the substance P-induced vasodilatation (Figure 3)

Destruction of adrenergic nerves in the hypothalamus with 6-OHDA abolished the vasodilatation caused by 500 ng substance P; 20 μg of the β-adrenoceptor blocker, propranolol, also blocked the substance P-induced vasodilatation. In addition, the intravenous injection of phenoxybenzamine (2.5 mg) also blocked the vasodilatation. Phenoxybenzamine was injected intravenously to avoid any pH effect of the solution on HBF.

Evidence that substance P exerts its effect via the intracerebral noradrenergic pathway (INP)

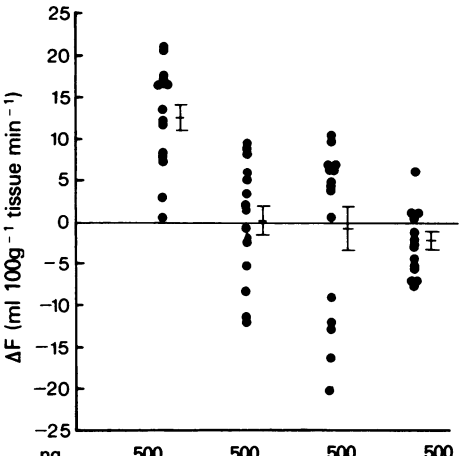
Stimulation of the INP has been shown previously to produce an increase in HBF similar to that produced by substance P (Mitchell *et al.*, 1978). Destruction of the pathway in the reticular formation abolishes the effect of substance P on HBF (Figure 4).

The INP is thought to cause a vasodilatation secondary to an increase in neuronal metabolism and a decrease in local pH. The evidence in favour of this hypothesis is the fact that barbiturate which has no effect on blood vessels (Mitchell *et al.*, 1978) or axons (Richards, 1972), but does depress neurones (Goodman & Gillman, 1965), abolishes the vasodilator effect of INP stimulation (Mitchell *et al.*, 1978). In addition, bicarbonate which would tend to buffer the fall in local pH, abolishes the vasodilatation due to INP stimulation (Klugman *et al.*, 1980).



ng	500 SP	500 SP + 300μg 6-OHDA	500 SP + 20μg Pro-pranolol	500 SP i.h. + 2.5mg Phenoxy-benzamine
	i.h.	i.h.	i.h.	i.h.
No. of Trials	15	15	15	20
x	12.7	1.1	1.3	0.15
s.e. mean	1.6	2.5	2.2	2.8
P	<0.001	NS	NS	NS

Figure 3 The effect of adrenoceptor blockade on a substance P (SP)-induced hypothalamic vasodilatation. Note that destruction of adrenergic nerves in the hypothalamus by 6-hydroxydopamine (6-OHDA) abolishes the vasodilatation. The β-adrenoceptor antagonist, propranolol, and the α-adrenoceptor antagonist, phenoxybenzamine, abolish the increase in hypothalamic blood flow due to substance P.



ng	500 SP	500 SP + 300μg 6-OHDA R.F.	500 SP + 60μg Barbiturate i.h.	500 SP + 40mM HCO_3 i.h.
No. of Trials	15	15	15	15
x	12.7	0.3	−0.6	−2.0
s.e. mean	1.6	1.8	2.7	1.1
P	<0.001	NS	NS	NS

Figure 4 Evidence that the intracerebral noradrenergic pathway (INP) mediates the hypothalamic vasodilatation due to substance P (SP). Destruction of the pathway in the reticular formation (R.F.) by 6-hydroxydopamine (6-OHDA), abolishes the vasodilator effect of substance P in the hypothalamus. Barbiturate and HCO_3^-, both known to block the vasodilator effects of INP stimulation, also block the vasodilator effects of substance P.

Figure 4 shows that both barbiturate (60 μg) and HCO_3^- (40 mM) abolish the vasodilator effect of substance P.

Discussion

Flows on the control side of the hypothalamus were constant in individual rabbits. There was no disruption of hypothalamic tissue on light microscopy in animals used in up to four experiments (Cranston & Rosendorff, 1971) and no animal was used more than three times in this study. The preservation of autoregulation is considered a sensitive index of vasomotor integrity and is maintained in the hypothalamus, by this technique, for a range of mean arterial blood pressure of 41 to 140 mmHg (Cranston & Rosendorff, 1971). Further, CO_2 responsiveness of HBF is maintained (Cranston & Rosendorff, 1971; Cameron & Caronna, 1976). In addition, blood pressures, blood gases and pH did not change significantly. Changes in HBF on the test side relative to the control side are therefore a reflection of the effects of the local injections of substance P. Further, this effect is dependent on the configuration of the substance P molecule since injection of the 11 constituent amino-acids was without effect on HBF.

A role for substance P in the local control of cerebral blood flow is suggested by the fact that immunohistochemistry indicates an innervation of cerebral blood vessels by substance P-containing nerves (Chan-Palay, 1977). In addition, a dilator action of substance P via perivascular axonal networks in the spinal cord, has been postulated (Barber, Vaughn, Slemmon, Salvaterra, Roberts & Leeman, 1979). This paper is the first account of a vascular action of substance P in the brain. In common with the vasodilatation reported for other vascular beds (von Euler & Gaddum, 1931; Lofstrom *et al.*, 1965; Maxwell, 1968; Hallberg & Pernow, 1975; Eklund *et al.*, 1977; Losay *et al.*, 1977; Lembeck & Holzer, 1979), the brain vasculature dilates in the presence of exogenous substance P.

Our results also suggest that the action of substance P is not direct. The release of acetylcholine appears to be essential to the effect of substance P as the cholinoceptor antagonists, atropine and mecamylamine, both abolish the vasodilatation due to substance P. One explanation for this blockade is that the cholinoceptor antagonists are occupying substance P receptors, but there is no evidence in the literature to suggest that cholinoceptor antagonists and substance P receptors interact in this way. An interaction also seems unlikely in view of the difference in the shape and molecular mass of substance P and cholinoceptor antagonists. Resolution of this possibility nonetheless depends on the discovery of a specific substance P receptor antagonist.

Another explanation for our results is that there is a true substance P-cholinergic interaction. An interaction between substance P and acetylcholine to produce hypothalamic vasodilatation is suggested by their close association in the brain. Cuello, Emson, Del Fiaco, Gale, Iverson, Jessel, Kanazawa, Paxinos & Quik (1978) have postulated the existence of projections from substance P-containing neurones on to acetylcholine and dopamine-containing neurones. The hypothalamus is rich in acetylcholine (Phillis, 1970) and substance P (Mroz *et al.*, 1977) and immunohistochemical studies on the subcellular distribution of acetylcholine and substance P have shown a close association of locations for these two agents (Kataska, 1962; Ryall, 1964). Recent work has shown that acetylcholine efflux from neurones in the hippocampus and brain stem is increased by the intraventricular injection of substance P (Malthe-Sorenssen *et al.*, 1978). However, if such an interaction is taking place, the actual nature of the cholinoceptor involved is not clear. The possibility that the receptor involved may be an intermediate cholinoceptor has been suggested previously (Klugman *et al.*, 1980), as the vasodilatation produced by stimulation of the INP can also be blocked by both atropine and mecamylamine (Klugman *et al.*, 1980).

The concept of an intermediate cholinoceptor in the CNS combining nicotinic and muscarinic properties was first suggested in 1975 (Bird & Aghajanian, 1975). It has since been suggested that the identification of cholinoceptors in the CNS appears much more complex than in the periphery (Giorguiff, Le Floc'h, Glowinski & Besson, 1976). These authors have shown, for example, that the spontaneous release of dopamine from striatal dopaminergic terminals in the rat is inhibited by both muscarinic and nicotinic cholinoceptor antagonists. Experiments to characterize fully the cholinoceptor involved in the interaction with noradrenaline in the hypothalamus are now being done.

The fact that the substance P vasodilatation is abolished by adrenoceptor antagonists as well as cholinoceptor antagonists is also consistent with the idea that an adrenergic system is involved in the vasodilator mechanism. The substance P vasodilatation is blocked by 6-OHDA, the α-adrenoceptor antagonist, phenoxybenzamine and the β-adrenoceptor antagonist, propranolol. The blockade of the response by both α- and β-adrenoceptor antagonists further suggests that the mechanism involves neurones. Microiontophoretic studies have indicated that central nervous system adrenoceptors combine the properties of both α- and β-adrenoceptors and can be blocked by both α- and β-adrenoceptor antagonists, although more consistently with β-adrenoceptor antagonists (Johnson, Roberts, Sobieszek & Straughan, 1969; Brawley & Johnson, 1973). In addition, the adrenoceptor antagonists, phenoxybenzamine and propranolol, have been shown to block the vasodilator effect of direct electrical stimulation of the INP (Mitchell *et al.*, 1978). It is possible therefore that substance P causes the release of noradrenaline and that this in turn acts on cholinergic nerves to produce the vasodilatation but this explanation is unlikely in view of the relative lack of experimental evidence of a noradrenergic mediation of acetylcholine release. However, evidence for a mediation of noradrenaline release by acetylcholine is abundant (Westfall, 1977). Further, exogenous cholinoceptor agonists may cause a hypothalamic vasodilatation, an effect also blocked by 6-OHDA and by propranolol (Klugman *et al.*, 1979). Thus the effects of substance P release are likely to be secondary to the release of acetylcholine, a conclusion supported by the findings that substance P has been shown to increase the turnover of brain noradrenaline (Carlsson, Magnusson, Fisher, Chang & Folkers, 1977) and to stimulate dopaminergic pathways (Michelot, Leviel, Giorguieff-Chesselet, Cheramy & Glowinski, 1979).

Our results also provide evidence that the adrenergic pathway involved in this response is the INP (destruction of the INP in the reticular formation abolishes the vasodilator effect of substance P in the hypothalamus). In addition, barbiturate, propranolol and phenoxybenzamine which abolish the effect of INP stimulation (Mitchell *et al.*, 1978) also abolish the substance P vasodilatation. Finally, bicarbonate, which has been shown to block the vasodilatation due to INP stimulation (Klugman *et al.*, 1980), also blocks the substance P response.

The origin of the cholinergic pathway involved in the substance P response is less certain. There is both histological and physiological evidence for an ascending cholinergic system, arising in the reticular formation and passing through the midbrain to the cerebral cortex (Krnjević, 1969). Although this is a diffuse system, its pathways parallel those of the INP and for this reason it is the likely source of a cholinergic influence on the INP. Another source of cholinergic

nerves is the VIIth cranial nerve, which carries vasodilator fibres to the meningeal blood vessels (Chorobski & Penfield, 1932) and the nucleus of which lies close to the origin of the INP.

In summary, our data suggest that substance P has a vasodilator effect on hypothalamic blood vessels. This effect is unlikely to be a direct one on blood vessels as it is abolished by both adrenoceptor and cholinoceptor blockade, but it is likely to be due to the presence of substance P specific receptors since the substance P amino-acid mixture is without effect. The vasodilatation is probably a result of substance P-mediated release of acetylcholine which in turn stimulates the vasodilator intracerebral noradrenergic pathway.

This indirect action of substance P on the hypothalamic circulation is in contrast to its direct vasodilator action on blood vessels in the periphery.

This work was supported by the Atomic Energy Board and the South African Medical Research Council. G.M. is the recipient of a University Council Research grant, K.P.K. is an M.R.C. research scholar and a 'Sailor' Malan Memorial research fellow.

References

BARBER, R.P. VAUGHN, J.E., SLEMMON, J.R., SALVATERRA, P.M., ROBERTS, E. & LEEMAN, S.E. (1979). The origin, distribution and synaptic relationships of substance P axons in rat spinal cord. *J. comp. Neurol.*, **184**, 331–351.

BIRD S. J. & AGHAJANIAN, G.K. (1975). Microiontophoretic study of the cholinergic pharmacology of hippocampal pyramidal cells. *Neurosci. Abstr.* **1**, (5th Annual Meeting of the Society for Neuroscience) 387.

BRAWLEY, P. & JOHNSON, E.S. (1973). Noradrenaline excitations: neuronal not vascular. *Nature, New Biol.* **242**, 245–246.

CAMERON, I.R. & CARONNA, J. (1976). The effect of local changes in potassium and bicarbonate concentration on hypothalamic blood flow in the rabbit. *J. Physiol.*, **262**, 415–430.

CARLSSON, A., MAGNUSSON, T., FISHER G. H., CHANG D. & FOLKERS, K. (1977). Effect of synthetic substance P on central monaminergic mechanisms. In *Substance P.* ed von Euler, U.S. & Pernow, B. pp. 201–205. New York: Raven Press.

CHAN-PALAY, V. (1977). Innervation of cerebral blood vessels by norepinephrine, indoleamine, substance P and neurotensin fibres and the leptomeningeal indoleamine axons. Their roles in vasomotor activity and local alterations of brain blood composition. In *Neurogenic Control of the Brain Circulation.* ed. Owman, C. & Edvinsson, L. pp. 39–54. Oxford: Pergamon Press.

CHOROBSKI, J. & PENFIELD, W. (1932). Cerebral vasodilator nerves and their pathway from the medulla oblongata. *Arch. Neurol. Psychiat*, **28**, 1257–1289.

CRANSTON, W.I. & ROSENDORFF, C. (1971). Local blood flow, cerebrovascular autoregulation and carbon dioxide responsiveness in the rabbit hypothalamus. *J. Physiol.*, **215**, 577–590.

CUELLO, A.C., EMSON, P., DEL FIACCO, M., GALE, J., IVERSEN, L.L., JESSEL, T. M., KANAZAWA, I., PAXINOS, G. & QUIK, M. (1978). Distribution and release of substance P in the central nervous system. In *Centrally Acting Peptides.* ed. Hughes, M. pp. 135–155. London: MacMillan Press.

EKLUND, B., JOGESTRAND, T. & PERNOW, B. (1977). Effect of substance P on resistance and capacitance vessels in the human forearm. In *Substance P*, ed. von Euler, U.S. & Pernow, B. pp. 275–285. New York: Raven Press.

GIORGUIEFF, M.F., LE FLOC'H, M.L., GLOWINSKI, J. & BESSON, M.J. (1976). Involvement of cholinergic presynaptic receptors of nicotinic and muscarinic types in the control of the spontaneous release of dopamine from striatal dopaminergic terminals in the rat. *J. Pharmac. exp. Ther.*, **200**, 535–544.

GOODMAN L.S. & GILLMAN, A. (1965). *The Pharmacological Basis of Therapeutics.* New York: MacMillan.

HALLBERG, D. & PERNOW, B. (1975). Effect of substance P on various vascular beds in the dog. *Acta. Physiol. scand.*, **93**, 277–285.

HEDQVIST, P. & VON EULER U.S. (1977). Effects of substance P on some autonomic neuroeffector junctions. In *Substance P.* ed. von Euler, U.S. & Pernow, B. pp. 89–95. New York: Raven Press.

HOLZER, P. & LEMBECK, F. (1980). Neurally mediated contraction of ileal longitudinal muscle by substance P. *Neurosci. Letts*, (in press).

JOHNSON, F.S., ROBERTS, M.H.T., SOBIESZEK, A. & STRAUGHAN, D.W. (1969). Noradrenaline sensitive cells in cat cerebral cortex. *Int. J. Neuropharmac.*, **8**, 549–566.

KATASKA, K. (1962). The subcellular distribution of substance P in nervous tissue. *Jap. J. Physiol.*, **12**, 81–96.

KLUGMAN, K., MITCHELL, G. & ROSENDORFF, C. (1979). Evidence for an indirect cholinergic regulation of blood flow in the hypothalamus of conscious rabbits. *Br. J. Pharmac.*, **66**, 217–221.

KLUGMAN, K.P., MITCHELL, G. & ROSENDORFF, C. (1980). Cholinergic regulation of intracerebral noradrenergic pathway-induced hypothalamic vasodilation. *Stroke*, (in press).

KRNJEVIĆ, K. (1969). Central cholinergic pathways. *Fedn Proc.*, **29**, 113–120.

LEMBECK, F. & HOLZER, P. (1979). Substance P as neurogenic mediator of antidromic vasodilatation and neurogenic plasma extravasation. *Naunyn-Schmiedebergs Arch. Pharmac.*, **310**, 175–183.

LOFSTROM, B., PERNOW, B. & WAHREN, J. (1965). Vasodilating action of substance P in the human forearm. *Acta. physiol. scand.*, **63**, 311–324.

LOSAY, J., MROZ, E.A., TREGEAR, G.W., LEEMAN, S.E. & GAMBLE, W.J. (1977). Action of substance P on the coronary blood flow in the isolated dog heart. In *Substance P.* ed. von Euler, U.S. & Pernow, B. pp. 287–293. New York: Raven Press.

MALTHE-SORENSSEN, D., CHENEX, S.L. & COSTA, E. (1978).

Modulation of acetylcholine metabolism in the hippocampal cholinergic pathway by intraseptally injected substance P. *J. Pharmac. exp. Ther.*, **206**, 21–28.

MAXWELL, G.M. (1968). Actions of substance P on the general, pulmonary and coronary haemodynamics and metabolism of intact dogs. *Br. J. Pharmac.*, **32**, 514–522.

MICHELOT, R., LEVIEL, V., GIORGUIEFF-CHESSELET, M.F., CHERAMY, A. & GLOWINSKI, J. (1979). Effects of the unilateral nigral modulation of substance P transmission on the activity of the two nigro-striatal dopaminergic pathways. *Life Sci.*, **24**, 715–724.

MITCHELL, G., MITCHELL, D. & ROSENDORFF, C. (1978). The vasodilator mechanism of the intracerebral (non-sympathetic) adrenergic pathway. *Cardiovasc Res.*, **12**, 42–48.

MONNIER, M. & GANGLOFF, H. (1961). *Atlas for Stereotaxic Brain Research on the Conscious Rabbit.* London: Elsevier.

MROZ, E.A., BROWNSTEIN, M.J. & LEEMAN, S.E. (1977). Distribution of immunoassayable substance P in the rat brain: Evidence for the existence of Substance P-containing tracts. In *Substance P.* ed. von Euler, U.S. & Pernow, B. pp. 147–154. New York: Raven Press.

PHILLIS, J.W. (1970). *The Pharmacology of Synapses.* p. 10. Oxford: Pergamon Press.

RICHARDS, C.D. (1972). On the mechanism of barbiturate anaesthesia. *J. Physiol.*, **227**, 749–767.

ROSENDORFF, C. (1972). The measurement of local cerebral blood flow and the effect of amines. In *Progress in Brain Research.* ed. Meyer, J.S. & Schade, J.P. pp. 115–156. Vol. 35, Amsterdam: Elsevier Publishing Co.

ROSENDORFF, C. & LUFF, R.H. (1970). An indirect method for the determination of the tissue blood partition coefficient for 133-xenon. *J. appl. Physiol.*, **29**, 713–716.

RYALL, R. W. (1964). The subcellular distribution of substance P, acetylcholine, serotonin and glutamic acid in brain homogenates. *J. Neurochem.*, **11**, 131–145.

UNGERSTEDT, U. (1971). Use of intracerebral injections of 6-hydroxydopamine as a tool for morphological and functional studies on central catecholamine neurons. In *6-hydroxydopamine and Catecholamine Neurons.* ed. Malmfors, T. & Thoenen, H. pp. 315–332. Amsterdam: North-Holland Publ. Co.

VON EULER, U.S. & GADDUM, J.H. (1931). An unidentified depressor substance in certain tissue extracts. *J. Physiol.*, **72**, 74–87.

WESTFALL, T.C. (1977). Local regulation of adrenergic neurotransmission. *Physiol. Rev.* **57**, 659–728.

(Received April 8, 1980.
Revised June 25, 1980.)