J. Pharm. Pharmacol. 1990, 42: 135–138
Communicated May 30, 1989

Arecoline, but not haloperidol, induces changes in the permeability of the blood-brain barrier in the rat

A. SAIJA, P. PRINCI*, R. DE PASQUALE, G. COSTA†, *Department Farmaco-Biologico, School of Pharmacy, and *Centro Interdipartimentale di Informazione Farmaco-Tossicologica, University of Messina; †Institute of Pharmacology, School of Pharmacy, University of Camerino, Italy*

Abstract—The aim of the present study was to investigate the existence of alterations of the blood-brain barrier (BBB) permeability in rats injected with centrally acting drugs, by calculating a unidirectional blood-to-brain transfer constant (K_i) for the circulating tracer [^{14}C]-α-aminoisobutyric acid. The intraperitoneal (i.p.) injection of the dopaminergic antagonist haloperidol (1 mg kg^{-1}) did not modify the regional BBB permeability. When the cholinomimetic agent arecoline hydrobromide (6·25 mg kg^{-1}) was injected i.p. into methylatropine-pretreated rats, it induced a significant decrease of K_i values within the frontal cortex, parietal cortex, striatum and brain-stem. Our findings emphasize two concepts: (1) centrally acting drugs, such as arecoline, can induce changes in the BBB permeability, through several mechanisms; (2) there is no predictable correlation of drug stimulation of specific brain neuronal pathways and changes in the permeability of the BBB.

The term 'blood-brain barrier' (BBB) has been used to account for the restricted movement of solutes between the vascular and the cerebral compartments. The different anatomical features as well as the various enzymatic and biochemical energy-dependent processes that constitute the BBB system have been reviewed recently (Betz & Goldstein 1984; Pardridge 1987, 1988). Clearly the structural and functional integrity of BBB is essential to normal neurological function.

It is now well known that different processes involved in the BBB can be selectively and temporarily altered by experimental means, as by administration of vasoactive drugs or peptides (Edvinsson 1982; Pardridge 1987). Recent research has shown that neuronal circuits within the central nervous system (CNS) directly influence various aspects of cerebral microvascular function (Burnstock 1985; Palmer 1986; Kobayashi et al 1986), and focus on determining the exact anatomical site of this regulation and the precise mechanism of action. Anyway, there is a paucity of data pertaining to quantitative estimates of changes in the permeability of the BBB induced by drugs acting on the CNS. In this paper, we report our findings regarding the effects of two drugs which selectively modify functional activity of brain neuronal pathways, arecoline (a cholinomimetic acting at muscarinic and nicotinic sites) and haloperidol (a dopaminergic antagonist), on the BBB permeability in the rat. In our experiments, determination of the permeability of the BBB involved the use of [^{14}C]α-aminoisobutyric acid ([^{14}C]AIB), a small molecular weight radiolabelled aminoacid, which can cross the BBB and allows the precise localization and quantitative expression of barrier changes (Gross et al 1982; Tyson et al 1982; Blasberg et al 1983; Picozzi et al 1985; Saija et al 1987).

Materials and methods

Animal preparation. Male Wistar rats, weighing 230–250 g, were maintained on a 12 h light/dark cycle (light on: 0600 h) with free access to food and water. On the day of the experiment, the animals were anaesthetized with pentobarbitone sodium (54 mg kg^{-1} intraperitoneally) and spontaneously breathed room air.

Correspondence to: R. De Pasquale, Department Farmaco-Biologico, School of Pharmacy, University of Messina, Contrada Annunziata, 98168 Messina, Italy.

Short PE20 polyethylene catheters were inserted in the left femoral vein and artery. Heparinized 0·9% NaCl (50 units mL^{-1}) was used to flush the lines. Before isotope injection and throughout the experimental period, arterial blood samples were withdrawn periodically for measurements of arterial blood gases and pH. Body temperature was maintained at 37°C by external heating. All experiments were carried out between 0900 and 1100 h.

Tracer administration. A bolus of 20 μCi of [^{14}C]AIB, in 0·25 mL injection volume, was injected intravenously. During the next 30 min nine timed arterial blood samples (80–100 μL) were withdrawn. A large blood volume was withdrawn at the end to measure the whole-blood isotope concentration. At the end of 30 min the animals were decapitated, their brains rapidly removed and dissected on a filter paper wetted with cold saline, in accordance with Paxinos & Watson (1982); the choroid plexus was carefully removed from the lateral ventricles and around the cerebellum. Tissue specimens were placed in preweighed vials, which were immediately reweighed; then the specimens were solubilized by adding 1 mL of Soluene 100 (Packard) and the vials incubated at 60°C overnight. Each vial was then filled with 4 mL of Hionic Fluor (Packard). Blood samples were centrifuged and 30 μL of plasma, diluted in 0·5 mL distilled water, was counted in 6 mL of Hionic Fluor. A 20 μL whole-blood sample was digested in 0·5 mL Soluene–ethanol (1 : 2) solution, blanched with 0·3 mL hydrogen peroxide (30% w/v) and counted in 6 mL Hionic Fluor. Beta counting was performed by a Packard PL Scintillation counter. Single samples quenching was monitored by the external standard method. Sample counts were corrected for background and quenching.

Regional blood volumes. Four animals for each experimental group were used to evaluate regional correction for the blood volume in the dissected brain samples. Blood volume was defined as the [^{14}C]sucrose space at 2 min after the intravenous injection of the tracer (Gross et al 1982; Picozzi et al 1985). A 10 μCi bolus of [^{14}C]sucrose was injected intravenously. Two minutes later a blood sample was collected and the animal killed. Brain samples and whole-blood were digested and counted in the similar way to [^{14}C]AIB.

Measurement of blood-to-brain AIB transfer constant. A unidirectional blood-to-brain transfer constant (K_i) for [^{14}C]AIB was calculated, for each brain region examined, by the following equation developed by Ohno et al (1978):

$$K_i = \frac{Ci(T) - VCb(T)}{\int_0^T Cp\,dt}$$

Where Ci(T) is the tissue concentration of the tracer at the end of the experiment (nCi g^{-1}), T is the duration of the experiment (min), Cp is the arterial plasma concentration of the tracer (nCi mL^{-1}), V is the residual regional blood volume (μL g^{-1}) and Cb(T) is the whole-blood tracer concentration (nCi mL^{-1}). Sucrose, a compound of very low permeability at the BBB, was used to calculate V as a ratio between the tracer concentration in

the brain and in the whole-blood according to the following equation:

$$V(sucr) = \frac{Ci(sucr)}{Cb(sucr)}$$

where V(sucr) is the cerebral blood volume ($\mu L\ g^{-1}$), Ci(sucr) is the tracer concentration ($nCi\ g^{-1}$) in the brain and Cb(sucr) is its concentration ($nCi\ mL^{-1}$) in the whole-blood.

Because K_i is related to the permeability-surface area product (PS) and blood-flow (F) by $PS = F \ln (1 - K_i/F)$, and $PS << F$ is a condition well satisfied for [^{14}C]AIB, $K_i \approx PS$ and can be expressed in terms of plasma clearance ($mL\ g^{-1}\ min^{-1}$).

Pharmacological treatments. Rats were randomized and assigned to one of the following groups (each of 6 animals): Groups 1 and 2: rats were injected intraperitoneally (i.p.), respectively, with haloperidol (1 mg kg^{-1}) or its vehicle (distilled water; 1 mL kg^{-1}), 45 min before [^{14}C]AIB injection; groups 3 and 4: rats were given i.p., respectively, arecoline hydrobromide (6·25 mg kg^{-1}) or its vehicle (distilled water, 1 mL kg^{-1}), 3 min before isotope administration; all animals in groups 3 and 4 were pretreated 7 min before arecoline or vehicle injection, with atropine methylbromide (4 mg kg^{-1} i.p.), a muscarinic antagonist which cannot cross the blood-brain barrier, so preventing only peripheral effects induced by cholinomimetic agents.

Drugs used. [^{14}C]AIB, Amersham, 59 mCi $mmol^{-1}$; [^{14}C] Sucrose, Amersham, 5–15 mCi $mmol^{-1}$; haloperidol (Serenase, Lusofarmaco, Italy); arecoline HBr and atropine methylbromide (Sigma); pentobarbital sodium (Serva, Heidelberg).

Statistical analysis. Results are expressed as mean ± s.e. of 6 determinations and compared by using Student's *t*-test for unpaired data.

Table 1. [^{14}C]AIB transfer constants (K_i) in male rats injected with vehicle (controls) or haloperidol (1 mg kg^{-1} i.p.). Each value is the mean ± s.e. of 6 determinations.

	K_i ($mL\ g^{-1}\ min^{-1} \times 10^{-3}$)	
Brain areas	Controls	Haloperidol
Cortex		
frontal	1·721 ± 0·106	1·927 ± 0·109
parietal	1·714 ± 0·132	1·631 ± 0·114
occipital	2·092 ± 0·074	2·032 ± 0·136
Striatum	1·001 ± 0·056	1·002 ± 0·043
Hippocampus	1·173 ± 0·099	1·058 ± 0·049
Hypothalamus	3·200 ± 0·108	3·216 ± 0·210
Cerebellum	1·688 ± 0·145	1·726 ± 0·038
Brain-stem	1·652 ± 0·140	1·726 ± 0·141

Table 2. [^{14}C]AIB transfer constants (K_i) in male rats injected with vehicle (controls) or arecoline hydrobromide (6·25 mg kg^{-1} i.p.). All animals were pretreated with atropine methylbromide (4 mg kg^{-1} i.p.). Each value is the mean ± s.e. of 6 determinations.

	K_i ($mL\ g^{-1}\ min^{-1} \times 10^{-3}$)	
Brain areas	Controls	Arecoline
Cortex		
frontal	1·785 ± 0·096	1·386 ± 0·101*
parietal	1·752 ± 0·103	1·389 ± 0·012*
occipital	1·996 ± 0·085	1·911 ± 0·133
Striatum	1·057 ± 0·049	0·901 ± 0·013*
Hippocampus	1·213 ± 0·101	1·231 ± 0·075
Hypothalamus	3·274 ± 0·115	3·547 ± 0·182
Cerebellum	1·628 ± 0·159	1·554 ± 0·045
Brain-stem	1·694 ± 0·131	1·308 ± 0·042*

* $P < 0·05$ versus controls.

Results

Consideration of [^{14}C]AIB technique. A critical component in this method is that a high concentration of intravascular tracer would result in a calculated K_i greater than the actual transfer constant. In our experiments, a correction for the intravascular tracer (by calculation of regional blood volumes) was introduced. On the other hand, accurate vascular volume estimates are difficult to derive because they are generated from different animals. In addition, the 30 min experimental time was chosen to minimize the effect of intravascular tracer on brain ^{14}C activity measured at the end of the experiment, and, also, the eventual brain-to-blood reflux of tracer.

The K_i values for all brain regions examined are consistent with those described by other authors (Gross et al 1982; Picozzi et al 1985) and previously obtained in our laboratory (Saija et al 1988a, b). Before isotope injection and throughout the experimental period, arterial blood gases and pH were within normal limits in all animal groups. The cerebral volume values, used as a correction for the tracer still within the vessels at the end of the experiments, did not differ significantly between experimental groups.

Finally, a brief comment on the employment of pentobarbitone anaesthesia in this series of experiments is needed. Previously we have demonstrated that the pentobarbitone-induced state of deep surgical anaesthesia, in the rat, is accompanied by a widespread and strong decrease of BBB permeability (Saija et al 1989). So, the possible effect of pentobarbitone anaesthesia cannot be taken into account to explain selective increases of [^{14}C]AIB K_i values.

Effect of haloperidol and arecoline administration on BBB permeability. As shown in Table 1, regional K_i values appeared unaffected following haloperidol injection in all brain areas examined. Data concerning the effect of arecoline administration on BBB permeability are reported in Table 2. The injection of methylatropine alone did not induce changes in BBB permeability characteristics (data not shown). When K_i values were calculated in rats given arecoline and premedicated with atropine methylbromide, they showed a statistically significant decrease within the frontal and temporal cortex, striatum and brain-stem, when compared with results obtained in control animals (injected with atropine methylbromide alone).

Discussion

For a long time it was thought that cerebral microvessels uniquely lacked reactivity to nervous and pharmacological stimuli. Subsequent studies revealed that a mixed population of receptors (including α_2-, β_2- and weak β_2-receptors and muscarinic cholinergic receptors) is present in the cerebral endothelium and modulates vasoregulatory mechanisms (Raichle et al 1976; Hartman et al 1980; Kobayashi et al 1981; Edvinsson 1982; Tuor et al 1986). Particularly, several in-vitro investigations are consistent with the possibility that there is a cholinergic innervation of cerebral small arterioles and capillaries which may be of intracerebral origin and could mediate vasodilatatory responses (Estrada & Krause 1982; Estrada et al 1983; Marchi &

Raiteri 1985; Dacey & Bassett 1987). The present in-vivo study shows that the cholinergic agonist arecoline, when injected systemically in atropine methylbromide-premedicated rats, significantly decreases the permeability of the BBB (that is the endothelium of cerebral blood vessels) in several brain areas. In contrast, Domer et al (1983) showed that acetylcholine causes an increase in the BBB permeability to sodium pertechnetate in normotensive rats; these results were carried out in animals not pretreated with atropine methylbromide, so that they might be related to acetylcholine-induced direct and/or indirect peripheral effects (for example, the decrease in the systemic blood pressure). In addition, we have demonstrated that the systemic injection of the dopaminergic blocking agent haloperidol does not produce changes in the local BBB permeability; this finding is in keeping with data by Sankar et al (1981) showing that haloperidol does not modify the extravasation of protein in the brain of normotensive and hypertensive rats.

Several mechanisms are known to alter BBB characteristics, including hypertension and anoxia. However, none of these mechanisms may be invoked to explain the increase in BBB permeability following arecoline administration. In fact, arecoline can increase arterial blood pressure in methylatropine premedicated animals; but evidence has been found for a dissociation between change in systemic blood pressure and change in the BBB permeability (Domer et al 1983). In addition, hypotension (also severe) was shown not to alter BBB characteristics (Domer et al 1985), while only a strong and rapid rise of blood pressure can increase the BBB permeability (Ellison et al 1985). Endothelial cells of BBB may be damaged by prolonged anoxia (Petito et al 1982) but in our experiments, throughout the experimental period, rats continued to have good colour and robust respiration, so that they did not suffer profound anoxia; in addition, no significant difference in blood Po_2, Pco_2 and pH was observed between control and experimental groups. Finally, the existence of a relationship between alterations of BBB permeability and arecoline-induced changes of cerebral blood flow may be excluded, because, in this mathematic model, the K_i for [^{14}C]AIB is independent of cerebral blood flow values (see Materials and methods). The findings presented herein emphasize two concepts; (1) drugs acting on the CNS, such as arecoline, can induce changes in the BBB permeability; (2) on the other hand, there is no predictable correlation between drug stimulation of specific neuronal pathways and changes in the permeability of BBB. In fact, the BBB permeability appears unaffected when challenged by haloperidol, at a dose (1 mg kg^{-1} i.p.) capable of producing a marked reduction in cerebral metabolism (blocking dopaminergic receptors) (Pizzolato et al 1985). On the contrary, arecoline (6·25 mg kg^{-1} i.p.) can elicit a significant elevation of regional cerebral metabolism by acting directly at muscarinic receptors (Soncrant et al 1985), while significantly decreasing the BBB local permeability within several cerebral regions. Thus, it is possible to speculate that centrally acting drugs induce modifications of BBB permeability through different (and perhaps concomitant) mechanisms: (1) by direct action of the drug at the level of receptors present in the cerebral capillary endothelium and subserving vasoregulatory responses; (2) by drug-induced changes in the neurogenic component controlling BBB permeability (as a consequence of an altered functional activity of cerebral neuronal circuits) and/or in the release of vasoactive substances.

In conclusion, our present study corroborates previous results from other workers indicating changes in the normal functioning of the BBB when challenged by centrally acting drugs (Sankar et al 1981, 1983; Domer et al 1983). Several mechanisms could be invoked to explain these findings; further understanding of such mechanisms could lead to new insights into both drug action at the BBB and drug delivery through this barrier.

References

Betz, A. L., Goldstein, G. W. (1984) Brain capillaries. Structure and Function. In: Lajtha, A. (ed.) Handbook of Neurochemistry. 2nd ed., Plenum, London, pp 465–484

Blasberg, R., Fenstermacher, J., Patlak, C. (1983) Transport of α-aminoisobutyric acid across brain capillary and cellular membranes. J. Cereb. Blood Flow Metab. 3: 8–32

Burnstock, G. (1985) Neurogenic control of cerebral circulation. Cephalalgia 5: 25–33

Dacey, R. G. Jr., Bassett, J. E. (1987) Cholinergic vasodilation of intracerebral arterioles in rats. Am. J. Physiol. 253: H1253–H1260

Domer, F. R., Boertje, S. B., Bing, E. G., Reddix, I. (1983) Histamine- and acetylcholine-induced changes in the permeability of the blood-brain barrier of normotensive and spontaneously hypertensive rats. Neuropharmacology 22: 615–619

Domer, F. R., Boertje, S. B., Sampson, J. B. (1985) Permeability of the blood-brain barrier is not changed by vasoactive doses of arachidonic acid and prostaglandins in rats. Res. Comm. Chem. Path. Pharmacol. 48: 27–37

Edvinsson, L. (1982) Vascular autonomic nerves and corresponding receptors in brain vessels. Pathol. Biol. 30: 261–268

Ellison, M. D., Povlishock, J. T., Hayes, R. L. (1985) Examination of the blood-brain barrier transfer of α-aminoisobutyric acid and horseradish peroxidase: regional alterations in blood-brain barrier function following acute hypertension. J. Cereb. Blood Flow Metab. 6: 471–480

Estrada, C., Krause, D. N. (1982) Muscarinic cholinergic receptor sites in cerebral blood vessels. J. Pharmacol. Exp. Ther. 221: 85–90

Estrada, C., Hamel, E., Krause, D. N. (1983) Biochemical evidence for cholinergic innervation of intracerebral blood vessels. Brain Res. 266: 261–270

Gross, P. M., Teasdale, G. M., Graham, D. I., Angerson, W. J., Harper, A. M. (1982) Intra-arterial histamine increases blood-brain transport in rats. Am. J. Physiol. 243: H307–H317

Hartman, B. K., Swanson, L. W., Raichle, M. E., Preskorn, S. H., Clark, H. B. (1980) Central adrenergic regulation of cerebral microvascular permeability and blood flow: anatomic and physiologic evidence. Advanc. Exp. Med. Biol. 131: 113–126

Kobayashi, H., Maoret, T., Ferrante, M., Spano, P., Trabucchi, M. (1981) Subtypes of beta-adrenergic receptors in rat cerebral microvessels. Brain Res. 220: 194–198

Kobayashi, H., Magnoni, M. S., Govoni, S., Izumi, F., Wada, A., Trabucchi, M. (1986) Neuronal control of brain microvessel function. Experientia 41: 427–434

Marchi, M., Raiteri, M. (1985) On the presence in the cerebral cortex of muscarinic receptor subtypes which differ in neuronal localization, function and pharmacological properties. J. Pharmacol. Exp. Ther. 235: 230–233

Ohno, K., Pettigrew, K., Rapoport, S. (1978) Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat. Am. J. Physiol. 235: 299–307

Palmer, G. C. (1986) Neurochemical coupled actions of transmitters in the microvasculature of the brain. Neurosci. Biobehav. Rev. 10: 79–101

Pardridge, W. M. (1987) The gate keeper: How molecules are screened for admission to the brain. The Sciences 27: 50–55

Pardridge, W. M. (1988) Recent advances in blood-brain barrier transport. Ann. Rev. Pharmacol. Toxicol. 28: 25–39

Paxinos, G., Watson, C. (1982) The rat brain in stereotaxic coordinates. Academic Press, New York

Petito, C. K., Pulsinelli, W. A., Jacobson, G., Plum, F. (1982) Edema and vascular permeability in cerebral ischemia: comparison between ischemic neuronal damage and infarction. J. Neuropathol. Exp. Neurol. 41: 423–426

Picozzi, P., Todd, N., Crockard, A. (1985) Regional blood-brain barrier permeability changes after restoration of blood flow in postischemic gerbil brains: a quantitative study. J. Cereb. Blood Flow Metab. 5: 10–16

Pizzolato, G., Soncrant, T. T., Holloway, H. W., Rapoport, S. I. (1985) Reduced metabolic response of the aged rat brain to haloperidol. J. Neurosci. 5: 2831–2838

Raichle, M. E., Eichling, J. O., Grubb, R. L., Hartman, B. K. (1976) Central noradrenergic regulation of brain microcirculation. In:

Pappine, H. M., Feindal, W. (eds) Dynamics of brain edema. Springer-Verlag, Berlin, pp 11–17
Saija, A, Princi, P., De Pasquale, R., Costa, G. (1987) Regional blood-brain barrier permeability changes in response to ACTH plasma levels modifications. Bull. Mol. Biol. Med. 12: 97–109
Saija, A., Princi, P., De Pasquale, R., Costa, G. (1988a) High intensity light exposure increases blood-brain barrier transport in rats. Pharmacol. Res. Comm. 20: 553–559
Saija, A., Princi, P., De Pasquale, R., Costa, G. (1988b) Circadian modifications of blood-brain barrier (BBB) permeability in the rat. Ibid. 20: 249–259
Saija, A., Princi, P., De Pasquale, R., Costa, G. (1989) Modifications of blood-brain barrier permeability and local cerebral metabolism in pentobarbital- and ketamine-anaesthetized rats. Neuropharmacol. 28: 997–1002
Sankar, R., Domer, F. R., Merine, D. S. (1981) Effects of dopaminergic and adrenergic blockage on amphetamine-induced extravasation of protein into the brain of normotensive and spontaneously hypertensive rats. Ibid. 20: 667–673
Sankar, R., Blossom, E., Clemons, K., Charles, P. (1983) Age-associated changes in the effects of amphetamine on the blood-brain barrier of rats. Neurobiol. Aging 4: 65–68
Soncrant, T. T., Holloway, H. W., Rapoport, S. I. (1985) Arecoline-induced elevations of regional cerebral metabolism in the conscious rat. Brain Res. 347: 205–216
Tuor, U. I., Edvinsson, L., McCulloch, J. (1986) Catecholamines and the relationship between cerebral blood flow and glucose use. Am. J. Physiol. 251: H824–H833
Tyson, G., Teasdale, G., Graham, D., McCulloch, J. (1982) Cerebrovascular permeability following MCA occlusion in the rat. J. Neurosurg. 57: 186–196

J. Pharm. Pharmacol. 1990, 42: 138–140
Communicated July 6, 1989

Effect of nicardipine on the relationship of renal blood flow and of renal vascular resistance to perfusion pressure in dog kidney

NOBUYUKI OGAWA, *Department of Pharmacology and Toxicology, Hatano Research Institute, Food and Drug Safety Center, 729–5 Ochiai, Hadano, Kanagawa, 257, Japan*

Abstract—The effect of nicardipine, a Ca channel blocker, on autoregulation of renal blood flow and perfusion pressure-vascular resistance relationship has been investigated in perfused kidneys of anaesthetized dogs. In control animals excellent autoregulation of renal blood flow and pressure-dependent elevation of vascular resistance were observed above 100 mmHg of perfusion pressure. However, intra-arterial infusion of nicardipine at doses of 3 and 10 $\mu g\ min^{-1}$ showed dose-dependent impairment of the autoregulatory response and of elevation of vascular resistance. Infusion of nicardipine (2·5 $\mu g\ min^{-1}$) into the renal artery also inhibited renal vasoconstriction induced by YC-170, a Ca channel activator. These results suggest that the inhibitory effect of nicardipine upon renal autoregulation may be due to its Ca^{2+} channel blocking action.

Ca channel blockers are useful as antihypertensive agents, because they relax vascular smooth muscle, thereby producing a reduction of peripheral vascular resistance. In the kidney, increase of renal vascular resistance is necessary for sustaining elevated systemic blood pressure (Guyton et al 1970). We have observed in dog kidney, that acute increase of perfusion pressure caused the elevation of renal vascular resistance (Ogawa & Ono 1986; Ogawa et al 1987). This phenomenon is based on an autoregulatory function in renal vasculature. Therefore, the effect of Ca channel blockers on autoregulatory function of renal vasculature is of interest.

In the present study, we examined the effect of nicardipine (Takenaka et al 1985) a potent Ca antagonist, on the autoregulation of renal blood flow, and analysed the relationship between perfusion pressure and renal vascular resistance. In addition, we have studied the effect of nicardipine on the decrease in renal blood flow induced by YC-170 (2-(2-pyridyl)ethyl 4-(*O*-chlorophenyl)-2,6-dimethyl-5-phenyl-carbamoyl-1,4-dihydropyridine-3-carboxylate), a Ca channel activator (Takenaka et al 1988).

Correspondence to: N. Ogawa, Department of Pharmacology and Toxicology, Hatano Research Institute, Food and Drug Safety Center, 729–5 Ochiai, Hadano, Kanagawa, 257, Japan.

Materials and methods

Preparation for autoregulation study. Five mongrel dogs of either sex, 14·3–24·0 kg, were used. Sedation was induced with morphine hydrochloride (2 mg kg^{-1} s.c.) and the animals anaesthetized with α-chloralose (40 mg kg^{-1} i.v.) and urethane (400 mg kg^{-1} i.v.). The left renal artery was exposed retroperitoneally, cannulated and perfused with blood from the carotid artery by means of a Harvard peristaltic pump (Model 1215). An initial dose of 500 units kg^{-1} of sodium heparin was given as anticoagulant. Perfusion pressure was regulated and adjusted by means of a Starling pneumatic resistance from which excess blood was conducted to the left jugular vein. Renal blood flow was measured with an electromagnetic flowmeter (Narco RT-500). Kidney perfusion pressure and femoral artery pressure were measured with transducers (Statham P23Db).

Renal blood flow was allowed to stabilize for 30 min at the basal perfusion pressure of 100 mmHg; perfusion pressure was then changed stepwise between 60 and 200 mmHg. Infusion of drug was started at the basal perfusion pressure of 100 mmHg, and pressure-flow relation was examined.

Preparation for studying the effect of nicardipine on YC-170-induced renal vasoconstriction. Five mongrel dogs of either sex, 10·0 to 15·4 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.) and artificially ventilated after the administration of decamethonium bromide (0·25 mg kg^{-1} i.v.) to induce paralysis of the skeletal muscle. Systemic blood pressure was measured with a pressure transducer (Statham P23Db) at the right brachial artery. A flank incision was made and the left kidney was exposed through a retroperitoneal approach. A non-cannulating electromagnetic flow probe (Narco RT-500, 2·5–3·0 mm in diameter) was placed around the renal artery adjacent to the aorta for measurement of renal blood flow. Nicardipine and YC-170 were infused into the renal artery proximal to the flow probe.

The first period was the control phase during which vehicle, 0·4 mL min^{-1}, was infused into the renal artery. Nicardipine was then infused into the renal artery at 2·5 $\mu g\ min^{-1}$ during the