EFFECT OF INDOMETHACIN (A PROSTAGLANDIN SYNTHETASE INHIBITOR) ON THE PERMEABILITY OF BLOOD-BRAIN AND BLOOD-CSF BARRIERS IN RAT

P.K. DEY, H.S. SHARMA AND K.S. RAO

Neurophysiology Research Unit,
Department of Physiology, Institute of Medical Sciences,
Banaras Hindu University, Varanasi-221 005

Summary: Effect of indomethacin on the permeability of blood-brain and blood-csf barriers to Evans blue, bromophenol blue and horseradish peroxidase (HRP) was studied by administering the drug through right internal carotid artery, right external jugular vein and through intraperitoneal route in rats. Increased permeability was observed on internal carotid arterial injection but not following jugular vein or intraperitoneal injection. This increased permeability of blood-brain and blood-csf barriers following injection of indomethacin into the internal carotid artery is associated with a steep and sustained rise in mean arterial blood pressure (MABP), a feature not observed in bilateral adrenalectromised animals.

Key words: indomethacin, adrenalectomy, hypertension, blood-brain barrier, blood-csf barrier

INTRODUCTION

Prostaglandin synthetase inhibitors (indomethacin, paracet mol and aspirin) are now clinically used mainly as antipyretic (6,7) and anti-inflammatory agents (10,22). It has recently been shown that inhibiton of prostaglandin synthesis by indomethacin administered orally or infused intravenously in man produces a rise in mean arterial blood pressure (MABP) (22,28). This hypertensive effect of indomethacin has also been confirmed in various animal studies (1,18,19,20). Hypertension is known to cause blood-brain barrier dysfunction to various protein tracers induced either, by administration of vasoactive substances in rats and cats (11,13,14,15), or by clamping of thoracic aorta in dogs (16), or by rapidly injecting 0.9% saline under high perfusion pressure into the internal carotid artery in rats (25).

Therefore, the present investigation has been undertaken in order to find out whether administration of indomethacin can induce a breakdown of cerebrovascular barriers, and if so, whether such breakdown is related with the hypertensive action of the drug. A preliminary communication in this regard has already been made (8).

MATERIALS AND METHODS

Albino rats (Charles-Foster and Holtzman strains) of either sex were used for experiments carried out under urethane anaesthesia (1.5 *g/kg*, ip) at temperatures 18° to 29°C.

Evans blue (Reanal, Hungary), bromophenol blue (BDH, England) (9), and horseradish peroxidase (HRP, Rz-0.6, Type I. Centron Research Laboratories, Bombay, India) were used as barrier tracer substances in this study.

A. Control group

Evans blue (2%, 3 *ml/kg*) was injected through right jugular vein in 6 rats (55-120 *g*) and into the right internal carotid artery towards brain (external carotid branch was ligated) in 5 rats (40-60 *g*). In 3 rats (40 *g*) bromophenol blue (0.8%, 3 *ml/kg*) was injected into right internal carotid artery. Horseradish peroxidase (2%, 0.5 *ml*) was injected in one rat (100 *g*) through right jugular vein.

5 min after dye injection, brain was perfused with 0.9% saline for 2-3 min followed by 10% formalin for 9 to 10 min. Then the brain was examined with naked eye for penatration of dye into the cerebral tissues and into cerebrospinal fluid.

The presence of HRP into the cerebral tissues was demonstrated according to the method of Malmgren and Olsson (21). The only modification done was that 40 μm thick frozen sections were collected in specially prepared vials having screw cap at the top and replacable nylon wire mesh at the bottom. Sections were then washed in 3-4 *ml* of the phosphate buffer, 3 times for ten min each. Incubation was done for 30 min in dark in a solution containing 10 *ml* of sodium cacodylate (BDH) pH 5.1, 20 *mg* of 3,3′ -diaminobenzidine (DAB, Sigma Chemical Co., USA) and 0.1 *ml* of 1% hydrogen peroxide. Sections were mounted unstained in gelatine alcohol mounting medium (4).

B. Indomethacin treated group

The action of indomethacin (Sigma Chemical Co., USA) on blood-brain and blood-csf barriers was studied by administering the drug (1 to 20 *mg/kg*) through right internal carotid route in 38 rats (body wt 100-300 *g*); 10 to 20 *mg/kg* through jugular vein in 6 rats (body wt 200-300 *g*) ; and 20 to 40 *mg/kg* intraperitoneally in 5 rats (body wt 90-200 *g*). Evans blue or HRP was injected through right jugular vein 5 min before indomethacin injection.

In other experiments the bromophenol blue dye was injected into the carotid artery after indomethacin injection to study the reversibility of the increased blood-brain and blood-csf barriers.

Recording of systemic arterial blood pressure

The blood pressure was recorded from the right carotid artery with the help of pressure transducer (Strain gauge, Stathum P23) which was connected to a chart recorder (Encardio-Rite 332, India).

Different doses of indomethacin were administered either through the internal carotid artery in 6 rats (body wt 200-300 *g*) by introducing another polythelene catheter in the right carotid artery (external carotid artery was tied), the tip of the catheter was directed towards the brain. or injected into the right jugular vein in 3 rats (body wt 300 *g*). In all these animals, Evans blue or HRP was injected to jugular vein 5 min before indomethacin.

Adrenalectomy

Bilateral adrenalectomy was done in 10 rats (body weight 200-300 *g*). Then they were divided in two groups. In one group (n=6), indomethacin (10 *mg/kg*) was injected into the right internal carotid artery after 15 to 38 min following adrenalectomy and permeability of cerebrovascular barriers were examined. In other group (n=4), the mean arterial blood pressure (MABP) was recorded from right common carotid artery following right internal carotid artery injection of the drug (10 *mg/kg*).

RESULTS

A. *Control studies*

Neither Evans blue nor bromophenol blue when injected through the internal carotid artery or jugular vein, was found in the cerebral tissues (Fig. 1A) or in the csf, as evident from the absence of blue staining of the cerebral parenchymal tissue or of the cerebroventricular walls (Table I). Similarly, horseradish peroxidase administered through jugular vein also did not cross the barriers as evident from the histochemical study (Fig 2A).

B. *Changes in the permeability of blood-brain and blood-csf barriers following indomethacin injection into the internal carotid artery*

Table I shows that in 6 rats, 1 *mg/kg* dose of indomethacin did not induce any change in the permeability of the barriers (either for Evans blue or HRP) examined 8 min after indomethacin injection.

But with a dose of 5 *mg/kg* in 5 rats, both the blood-brain and blood-csf barriers became permeable in two rats and in another two rats the blood-brain barrier only showed increased permeability 8 min after indomethacin (bromophenol blue dye was used as

barrier tracer). Usually the blue staining appeared in the ipsilateral cerebral hemisphere, mainly in the parieto-occipital region and in the pyriform area.

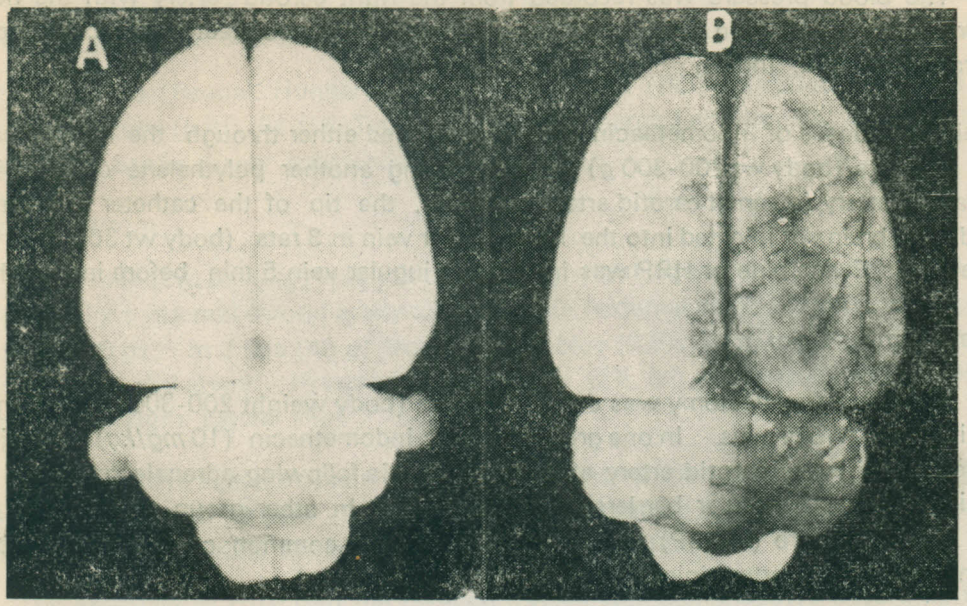

Fig. 1 : (A) Control rat brain showing the absence of Evans blue following its injection into right external jugular vein.

(B) Penetration of Evans blue mainly into ipsilateral cerebral and cerebellar cortical tissues following right intracarotid indomethacin (10 *mg/kg*) administration.

With 10 *mg/kg* dose of indomethacin, the increased permeability (Evans blue as barrier tracer) of blood-brain and blood-csf barriers were observed in 5 rats and only opening of blood-brain barrier in 3 rats 8 min after drug injection. The permeability was noted mainly around the pial vessels of ipsilateral parieto-occipital and pyriform area of cerebral cortex. Cerebellar cortex also became permeable in 3 rats. In another 3 rats, contralateral hemisphere also showed permeability along the mid line (Fig 1B). Right lateral ventricle (particularly anterior horn) was stained in most of the cases associated with occasional staining of caudate nucleus and dorsal surface of hippocampus. In several cases the thalamus, hypothalamus, collicular region were also stained.

With more higher doses of drug viz., 15 *mg/kg* (4 rats) and 20 *mg/kg* (2 rats), the extent of increase in blood-brain and blood-csf barrier permeability remained same as that of 10 *mg/kg*, examined after 6 to 10 min following the drug injection. In 2 rats, following right internal carotid artery injection, instead of right cerebral hemisphere, left cerebral hemisphere was found to be stained, possibly due to vascular anomaly.

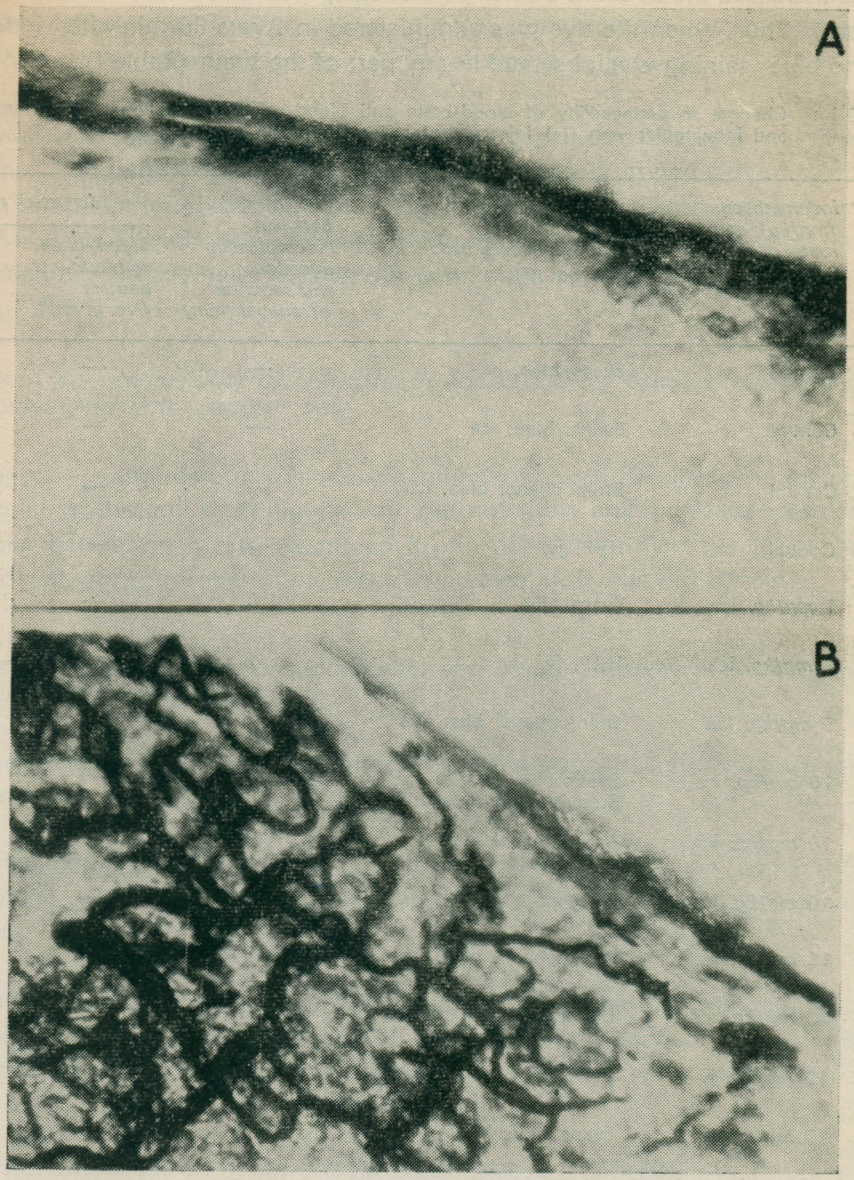

Fig. 2 : (A) Absence of extravasation of HRP tracer in control rat brain. HRP was injected through right jugular vein and animal was sacrificed 5 min after tracer injection (X 650).

(B) Exudation of HRP across cerebral blood vessels in parietal region of rat brain following intracarotid injection of 10 *mg/kg* indomethacin. HRP (10 *mg*/100 *g* body wt) was injected through right jugular vein 5 min before and animal was sacrificed 8 min after indomethacin injection respectively (X 650).

This increased blood-brain barrier permeability with indomethacin was observed to be reversible. Thus, when the dye was administered in 2 rats 30 min after indomethacin injection, no blue staining was observed in any part of the brain (Table I).

TABLE I : Changes in permeability of blood-brain and blood-csf barriers following intracarotid (ica) and intrajugular vein (jv) injection of indomethacin in rat.

Body wt. (g) range	*Indomethacin (mg/kg) and route of injection*	*Protein tracers used*	*Time interval between indomethacin injection and sacrifice of animal (min)*	*Increased permeability*	
				Blood–brain & blood–csf barriers (No. of rats)	*Blood–brain barrier only (No. of rats)*
55-120 (6)	Control	Evans blue, jv	—	—	—
40-60 (6)	Control	Evans, blue, ica	—	—	—
40 (3)	Control	Bromophenol blue, ica	—	—	—
100 (1)	Control	HRP, jv	—	—	—
140-150 (4)	1 *mg/kg*, ica	Evans blue, jv	8	—	—
140 (2)	1 *mg/kg*, ica	HRP, jv	8	—	—
160-200 (5)	5 *mg/kg*, ica	Bromophenol blue, ica	8	2	2
160-300 (16)	10 *mg/kg*, ica	Evans blue, jv Bromophenol blue, ica	8-10	7	8
200-260 (2)	10 *mg/kg*, ica	Bromophenol blue, ica	30	—	—
100-300 (3)	10 *mg/kg*, ica	HRP, jv	8	—	3
230-300 (4)	15 *mg/kg*, ica	Bromophenol blue, ica	6-10	3	1
100-240 (2)	20 *mg/kg*, ica	Brompophenol blue, ica	8-10	—	2
200-300 (5)	10 *mg/kg*, jv	Evans blue, jv	8	—	—
220 (1)	20 *mg/kg*, jv	Evans blue, jv	8	—	—
200-300 *Adrenal-ectomized (6)	10 *mg/kg*, ica	Evans blue, jv	8	—	—

*indomethacin was injected 15 to 38 min after bilateral adrenalectomy

NB : The figures in parenthesis indicate the number of animals

The pH of indomethacin solution (pH 8.4) had no effect on permeability as observed following administration of 3 *ml/kg* saline (pH 8.4) through the internal carotid artery in 3 rats. No increased permeability to dye was observed in these rats.

Entry of HRP across cerebral blood vessels was examined in 3 rats 8 min after indomethacin (10 *mg/kg*). Extensive brown reaction product of peroxidase was observed around large cerebral arterioles in all rats. The dilatation of cerebral vessels was evident. Peroxidase has just diffused out of vessels and confined around them since peroxidase in the vessel lumen has already been washed out with 0.9% saline during the process of brain fixation (Fig 2B).

C. *Changes in the permeability of blood-brain and blood-csf barriers following injection of indomethacin into jugular vein*

In contrast to intracarotid injection, administration of 10 *mg/kg* indomethacin to systemic circulation through right jugular vein did not show any increased permeability of cerebrovascular barriers in 5 rats, examined 8 min after drug injection (Table I). Even with a dose of 20 *mg/kg* in one rat, increase in permeability was not observed.

D. *Changes in the permeability of blood-brain and blood-csf barriers following intraperitoneal injection of indomethacin*

Intraperitoneal injection of indomethacin has no significant action on the permeability of cerebrovascular barriers.

Thus, following a very high dose (20 *mg/kg*) given in 3 rats, no penetration of the dye was observed whan brain was examined 15 min, 30 min and 50 min respectively after drug injection.

However, with a dose of 40 *mg/kg* (2 rats), right parieto-occipital region and pyriform area took mild stain examined 10 and 20 min after drug injection. In one rat, colliculer region had also took mild stain. Carebroventricular walls were not stained.

E. *Effect of indomethacin on mean arterial blood pressure (MABP)*

(a) *Intracarotid injection:* Intracarotid administration of indomethacin produced more or less a dose-dependent rise in MABP which was sustained for 10 to 15 min. The results are shown in Table II.

TABLE II : Changes in mean arterial blood pressure (MABP) following administration of indomethacin in rat.

Expt. No.	*Indomethacin (mg/kg) and route of injection a) ica; b) jv*	*MABP before indomethacin (mm Hg)*	*Peak rise in MABP after indomethacin (mm Hg)*	*Absolute rise (mm Hg)*	*Peak rise time after indomethacin (sec)*	*Maintenance of peak rise (sec)*	*Change in permeability of blood–brain barrier*
1	1 a	130	150	20	70	140	—
2	1 a	130	150	20	80	140	—
3	5 a	150	195	45	35	240	+ (Evans blue)
4	5 a	140	180	40	240	620	—
5	10 a	100	175	75	66	100	+ (HRP)
6	10 a	180	270	90	50	170	Not done
7	10 b	180	220	40	140	200	—
8	10 b	110	155	45	120	310	—
9	10 b	180	190	10	80	470	—

ica = internal carotid artery
jv = jugular vein
\+ = increased permeability
— = no change in permeability

A 20 mmHg rise in MABP was observed in 2 rats (130 mmHg to 150 mmHg) after 70 to 80 sec following indomethacin (1 *mg/kg*) injection, and the rise was maintained upto 140 sec. No penetration of the dye was observed in carebral tissues or ventricular walls examined 10 min after drug injection.

5 *mg/kg* indomethacin produced a rise of 45 mmHg (150 mmHg to 195 mmHg) after 35 sec in one rat and 40 mm Hg (140 mmHg to 180 mmHg) after 240 sec in another rat. Examination of brains 10 min after indomethacin injection showed permeation of Evans blue in right cerebral cortex and cerebellum in first rat, and this was absent in second rat.

10 *mg/kg* indomethacin injcted in 2 rats produced a sharp rise of 75 mm Hg (100 mmHg to 175 mmHg) in one rat and 90mmHg (180mmHg to 275 mmHg) in other rat. This rise of pressure was attained within 50 to 66 sec after injection and was maintained upto 100 and 170 sec respectively. HRP was injected in one rat 5min before indomethacin and the brain was prpcessed for peroxidase activity 10 min after drug injection. Extensive brown reaction product of HRP was observed around cerebral vessels.

(b) *Jugular vein injection:* When 10 *mg/kg* indomethacin was injection into jugular vein in 3 rats, only 10 to 45 mmHg rise of pressure occured after a latency of 80 to 140 sec following injection of indomethacin (Table II). In these rats, no increased permeability of cerebrovascular barriers was observed examined 8 min after drug injection.

F. *Changes in the permeability of blood-brain and blood-csf barriers following intracarotid injection of indomethacin after adrenalectomy*

Following acute bilateral adrenalectomy, the increased permeability of blood-brain and blood-csf barriers following intracarotid injection of indomethacin (10 *mg/kg*) was abolished as examined in 6 rats (Table I).

G. *Effect of indomethacin in mean arterial blood pressure (MABP) after adrenalectomy*

Bilateral adrenalectomy in 4 rats attenuated the magnitude and duration of rise in blood pressure following injection of indomethacin (10 *mg/kg*). Thus, only 35 to 40 mmHg rise in 3 rats and 70 mmHg rise in one rat were observed which returned to control levels within 35 to 80 sec (Table III).

TABLE III : Effect of indomethacin on mean arterial blood pressure (MABP) in bilaterally adrenalectomized rats.

Expt. No.	*Indomethacin (mg/kg) injected ica*	*Time interval of indomethacin injection after adrenalec–tomy (min)*	*MABP before indomethacin (mmHg)*	*MABP after indomethacin (mmHg)*	*Absolute rise (mmHg)*	*Peak rise time after indomethacin injection(sec)*	*Maintenance of peak rise (sec)*
1	10	40	160	200	40	10	1
2	10	35	180	250	70	5	1
3	10	30	100	140	40	5	5
4	10	35	100	135	35	5	5

Ica = internal carotid artery.

DISCUSSION

The present results show that indomethacin (5 to 20 *mg/kg*) injected into the internal carotid artery in rats significantly increases the permeability of cerebrovascular barriers to various protein tracers (Evans blue) bromophenol blue or HRP). This increased permeability was observed to occur within 6 to 10 min following indomethacin injection. Out of 30 rats, both blood-brain and blood-csf barriers were opened in 12 rats, while in 16 rats, only blood-brain barrier showed increased permeability to protein tracers. But such breakdown of cerebrovascular barriers was no longer observed when the protein tracers were injected into the carotid artery 30 min after indomethacin injection, which indicates that this opening of the barrier is reversible in nature. But the administration of indomethacin into systemic circulation through jugular vein did not show such breakdown of barriers to protein tracers.

This increased permeability of barriers may result from the rapid rise in blood pressure caused by imdomethacin injection. This possibility is envisaged from the various reports that indomethacin administration increases mean arterial blood pressure (MABP) in rabbits (1,5), dogs (20), rats (19) and in man (23,24,27). And several workers (11,13, 14,15,16,17) have observed that there exists a close relationship between the acute hypertension and the breakdown of cerebrovascular barriers. They have shown that steep rise in MABP in rats, cats, and dogs induced by vasoactive substances or by clamping of thoracic aorta, induce extravasation of protein tracers within 30 sec to 30 min after rise in blood pressure. They have pointed out t at it was not the total blood pressure rise which affected blood-brain barrier permeability, rather it was the intensity and rate of rise, which affected the permeability. For instance, a slow but big rise in blood pressure may not be associated with the leakage of protein tracers across the barriers. Our results also conform to these viewpoints. Thus, a sharp and sustained rise of 40 to 90 mmHg in MABP following intracar tid injection of indomethacin, was accompanied by extravasation of dyes or horseradish peroxidase. But when the rise in pressure was small as observed with 1 *mg/kg* indomethacin, or the rise was either slow or less following drug injection into jugular vein, or the rise is steep but occured only for a transient period as observed in bilateral adrenalectomized rats, no increased permeability to barrier tracers was observed.

These findings may be explained possibly on the basis of autoregulation of cerebral blood flow. Autoregulation comes into play within 30 sec to 120 sec following any rise in systemic arterial pressure (2,11,26). Thus if sufficient rise in mean arterial blood pressure (MABP) is rapidly attained and maintained before autoregulation begins to operate, the barrier functions may undergo modification, but if the rate of rise is slow then autoregulation can compensate the effects of such rise in MABP.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. J. Nagchaudhuri, Head of the Department of Physiology, Institute of Medical Sciences, B.H.U. for providing the laboratory facilities and State Council of Science & Technology, U.P. for partial financial assistance to this work.

REFERENCES

1. Anggard, E. and C. Larsson. Prostaglandin mediated hypotensive effects of arachidonic acid in the rabbit. *Acta Physiol. Scand. Suppl.*, **396** : 18, 1973.

2. Betz, E. Cerebral blood flow: Its measurement and regulation. *Physiol. Rev.*, **52** : 595-630, 1972.

3. Brightman, M.W., M. Hori, S.I. Rapoport, T.S. Reese and E. Westergaard. Osmotic opening of tight junctions in cerebral endothelium. *J. Comp. Neurol.*, **152** : 317-326, 1973.

4. Chan-Palay, V. *Cerabellar Dentate Nucleus*, p. 502, Springer-Verlag, New York, 1977.

5. Colina-Chourio, J., J.C. Mc Giff and A. Nasjletti. Development of high blood- pressure following inhibition of prostaglandin synethsis. *Fed. Proc.*, **34** : 368 (Abstract 841), 1975.

6. Dey, P.K., W. Feldberg and S. Wendlandt. Lipid A and prostaglandin. *J. Physiol. (Lond.)*., **239** : 102p-103p, 1974a.

7. Dey, P.K., W. Feldberg, K.P. Gupta, A.S. Milton and S. Wendlandt. Further studies on the role of prostaglandins in fever. *J. Physiol. (Lond.)*., **241** : 629-646, 1974b.

8. Dey, P.K. and H.S. Sharma. Effect of prostaglandin synthetase inhibitor on the permeability of blood-brain and blood-csf barriers. *Ind. J. Physiol. Pharmac.*, **22** : 177-179, 1978.

9. Feldberg, W. and K. Fleischhauer. Penetration of bromophenol blue from the perfused cerebral ventricles into the brain tissue. *J. Physiol. (Lond.)*, **150** : 451-462, 1960.

10. Flower, R., R. Gryglewski, K. Herbaczynska-Cedro and J.R. Vane. Effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nature (New Biology)*, **238** : 104-106, 1972.

11. Haggendal, E. and B. Johansson. On the pathophysiology of the increased cerebrovascular permeability in acute arterial hypertension in cats. *Acta Neurol. Scand.*, **48** : 265-270, 1972.

12. Haas, R.A., D.M. Robertson, H.B. Dinsdale and P.E. Davis. The role of adrenals in acute angiotensin-induced hypertension. In *"Blood Flow and Metabolism in the Brain"*. Eds. A.M. Harper., W.B. Jennett, J.D. Miller, J.O. Rowan, p. 524-528. Churchill Livingstone, New York, 1975.

13. Johansson, B. Brain barrier pathology in acute arterial hypertension. In: *"Transport phenomena in the nervous system"*, Eds. G. Levi., L. Battistin and A. Lajtha, 517-527. Plenum Press, New York, 1976.

14. Johansson, B. Effect of dexamethasone on protein extravasation in the brain in acute hypertension induced by amphetamine. *Acta Neurol. Scand.*, **57** : 180-185, 1978.

15. Johansson, B., C.L. Li, Y. Olsson and I. Klatzo. The effect of acute arterial hypertension on the blood-brain barrier to protein tracers. *Acta Neuropathol. (Berl.)*, **16** : 117-124, 1970.

16. Johansson, B. and L.E. Linder. Blood-brain barrier in acute arterial hypertension induced by clamping of thoracic aorta. *Acta Neurol. Scand.*, **50** : 360-365, 1974.

17. Johansson, B. and B.K. Siesjo. Brain energy metabolsim in angiotensin induced acute hypertension in rats. *Acta Physiol. Scand.*, **100** : 182-186, 1977.

18. Larsson, C. and F. Anggard. Arachidonic acid lowers and indomethacin increases the blood pressure of the rabbit. *J. Pharm. Pharmacol.*, **25** : 653-655, 1973.

19. Levy, J.V. Changes in systolic arterial blood pressure in normal and spontaneously hypertensive rats produced by acute administration of inhibitors of prostaglandin biosynthesis. *Prostagladings*, **13** : 153-160, 1977.

20. Lonigro, A.J., H.D. Itoskowitz, K. Crowshaw and J.C. McGiff. Dependency of renal blood flow on prostaglandin synthesis in the dog. *Circulat. Res.*, **32** : 712-716, 1973.

21. Malmgren, L. and Y. Olsson. A sensitive method for histochemical demonstration of horseradish peroxidase in neurons following retrograde axonal transport. *Brain Res.*, **148** : 279-294, 1978.

22. Moncada, S., S.H. Ferreira and J.R. Vane, Prostaglandins, aspirin-like drugs and the oedema of inflammation. *Nature (Lond.)*, **246** : 217-218, 1973.

23. Nowak, J., and A. Wennmalm. Influence of indomethacin and of prostaglandin E on total and regional blood flow in man. *Acta Physiol. Scand.*, **102** : 484-491, 1978.

24. Patak, R.V., B.K. Mookerjee, C.J. Bentzel, P.E. Hysert, M. Babej and J.B. Lee. Antagonism of the effects of furosemide by indomethacin in normal and hypertensive man. *Prostaglandins*, **10** : 649-459, 1975.

25. Rapela, C.E. and H.D. Green. Autoregulation of canine cerebral blood flow. *Circulat. Res.*, **15** *(Suppl. 1)* : 205-212, 1964.

26. Rapoport, S.I. and H.K. Thompson. Opening of the blood-brain barrier (BBB) by a pulse of hydrostatic pressure. *Biophys. J.*, **15** : 326a, 1975.

27. Wennmalm, A. Hypertensive effect of prostaglandin synthesis inhibitor indomethacin. *IRCS*, **2** : 1099, 1974.

28. Ylitalo, P., H. Vapaatalo, T. Metsa-Ketela and T. Pitakajarvi. Dependence of plsama renin activity on prostagladin excretion in essential hypertension. *Acta Physiol. Scand.*, **102** : 120-122, 1978.