cases, interburst frequency; mean intraburst frequency did not alter (figure 2). These responses resulted in an increase in the total activity in that afferent fibre. At higher intrasinus pressures, where activity was converted to a steady discharge, sympathetic stimulation no longer altered afferent activity.

Discussion. One possible explanation of these results concerns the distribution of baroreceptor endings in the carotid sinus. Some of these lie close to isolated smooth muscle cells near the medio-adventitial border[18]. Presumably in this location they would be subjected to mechanical responses of the smooth muscle cell. It is quite reasonable to suppose that in the sympathetically-denervated sinus region smooth muscle cells may undergo cyclic spontaneous contraction. In the isolated rat aortic strip we have observed spontaneous smooth muscle contractions whose frequency increased during norepinephrine administration. Presumably sympathetic stimulation would evoke comparable changes in contractile activity in the smooth muscle cells of the sinus wall, increasing the frequency and duration of bursting activity of suitably located baroreceptor afferents.

In several rabbit preparations we observed that a single pulse to the cervical sympathetic nerve was able to initiate a burst of activity in a B-type afferent held at subthreshold pressure. This response showed refractoriness, but virtually continuous afferent activity could be obtained at any subthreshold pressure by application of a single stimulus to the sympathetic nerve just after the refractory period had ended.

These sympathetic-generated bursts of activity could also have been secondary to activation of adjacent smooth muscle cells, or might have been a response to the direct effects of noradrenaline on the afferent endings. If we are to believe that the spontaneous bursting of these B-type afferents was a consequence of their location adjacent to smooth muscle cells, then it would seem more reasonable that sympathetic modulation of this activity was secondary to sympathetic influence on the smooth muscle. It appears, therefore, that a single stimulus to the cervical sympathetic nerve can trigger a smooth muscle cell to contract.

In summary, low threshold carotid sinus baroreceptor afferents (presumptive A-fibres) are generally not susceptible to sympathetic modulation. Some of these fibres demonstrate a bursting discharge at subthreshold pressures and this activity is augmented by sympathetic nerve stimulation. It is likely that these afferent fibres have endings closely associated with smooth muscle cells. The profound baroreflex effects of sympathetic nerve stimulation, or of the systemic infusion or local administration of catecholamines, must be due to the direct influence of catecholamines on the high threshold unmyelinated C-fibre afferents.

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A transbasisphenoidal approach for selective occlusion of the middle cerebral artery in rats

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Summary. The authors report a new model of focal cerebral ischemia following a selective occlusion of the middle cerebral artery in rats, without additional insults of hypoxia, hypotension and handling of carotid and vertebral arteries, as required in previously described models.

At present, several experimental models are available to investigate the phenomena related to cerebral ischemia. Particularly, with reference to focal cerebral ischemia, the most used models consist of: a) ligation of the common carotid artery in gerbils[1]; b) occlusion of the middle cerebral artery (MCA) by transorbital approach in squirrel monkeys[2], cats[3], dogs[4], baboons[5], rabbits[6]; c) occlusion of the MCA in rats by transpalatine approach[7]; d) embolization by platelet aggregates in rabbits[8], by moulded silicone cylinders in primates[9], by carbon microspheres in rats[10]. Small animals can be used in large numbers because of their low cost and ease of accommodation. Rats have commonly been used in these studies[7,10–15]; the disadvantages of some of these models are due to hypoxia and/or hypotension which are required, in addition to the vascular occlusion, for producing cerebral ischemia. Recently, Pulsinelli and Brierley[16] introduced a new model of bilateral hemispheric ischemia in unanesthetized rats. In this model, cerebral ischemia is obtained by occlusion of the vertebral and carotid arteries. In the present paper we are reporting a model of focal cerebral ischemia in rats, following a selective occlusion of the MCA by a transbasisphenoidal approach.

For development of this technique, 26 albino rats (Sprague-Dawley), weighing 250–300 g were utilized. 10 of these animals were used to provide an anatomical view of the surgical procedure and to attain an adequate level of microsurgical skill. 4 rats were used for parallel sham operations. The animal, under ether anesthesia, is placed on its back; after an accurate exposure, the trachea is cannulated directly by a 16-gauge needle. By exposure and catheterisation of the femoral artery, the mean arterial

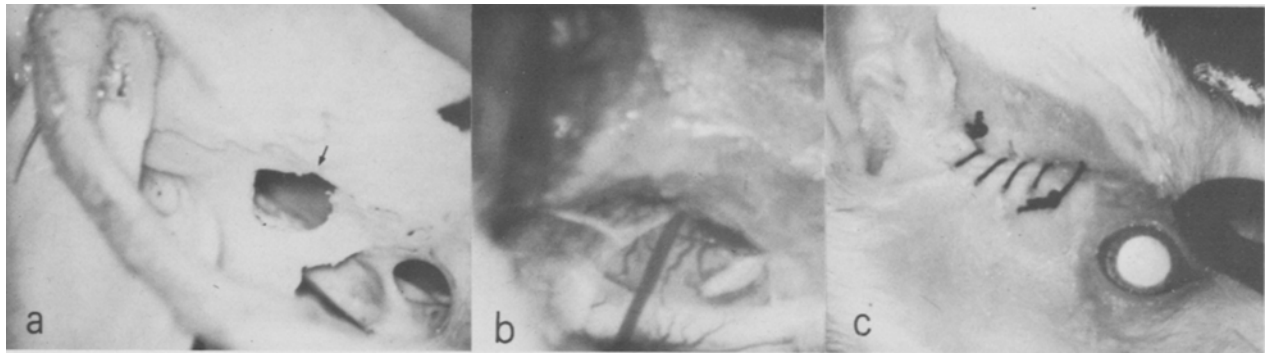

Fig. 1. *a* Skull of rat in operative position showing the cranial window (arrow); *b* intraoperative view showing the MCA crossing the olfactory tract; *c* animal's head fixed in the device; the surgical wound is sutured.

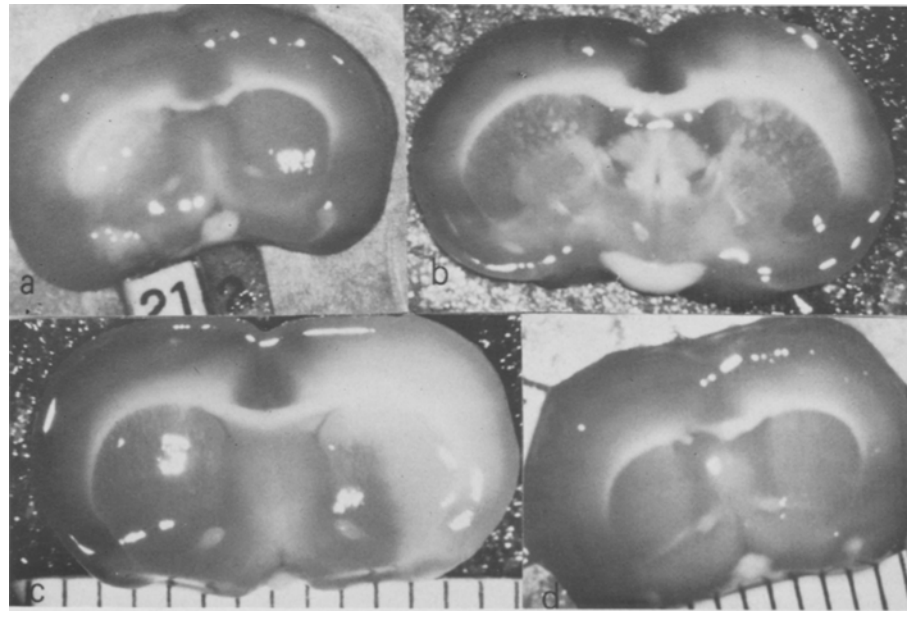

Fig. 2. Coronal slices of rat brains, perfused by Neutral Red, showing colourless (i.e. ischemic) areas; *a* in the basal ganglia, *b* in the cortical-subcortical region, *c* combined in the basal ganglia and cortical-subcortical region. *d* This slice does not reveal any colourless area.

pressure is monitored and blood levels of pO_2, pCO_2 and pH are measured as well during the surgical procedure. The animal's head is fixed in a device that allows its to be rotated about 70° to the left side. After shaving the right fronto-parieto-temporal region, a skin incision, about 8 mm in length, is made on the lateral border of the skull convexity. This site of incision is preferred to preserve the branches of the facial nerve; obviously, accidental damage of these branches would cause facial palsy and consequent keratitis. The fascia of the temporal muscle is then exposed retracting the surgical skin wound towards the temporal region. After transverse incision of this muscle is performed, 2 retractors are placed in order to expose the portion of the basisphenoid bone, located posteriorly to the optic foramen and anteriorly to the foramen ovale[17]. An operating microscope is needed for the subsequent surgical stages. A circular window of about 2 mm in diameter is cut by means of a dental drill, proximally to the orbital fissure (figure 1,a). After opening the underlying dura, the MCA is then exposed (figure 1,b). It is noteworthy that, in this region, the MCA is crossing the lateral olfactory tract. By bipolar coagulation, the main trunk of the artery is coagulated medially to the olfactory tract. The small bone flap is then replaced and, after removal of the retractors, a piece of gelfoam is placed deeply in the wound. The procedure is completed by applying a broad-spectrum antibiotic powder topically and closing the wound by continuous suture (figure 1,c). Lastly, the tracheal cannula and femoral catheter are removed. The overall procedure is simple and can be completed within about 30 min.

In one case only, the surgical procedure was interrupted by bleeding from the retro-orbital venous network; this animal was discarded from the study. In all other animals there was no intra-operative mortality; significant changes in mean arterial pressure, arterial pH and blood gas levels were not detected. Recovery from anesthesia occurred usually within about 10 min. No seizure was evidenced in 11 rats in which the surgical procedure was completed. 2 animals died 3 days after surgery. The observation of their brains revealed a large infarct with edema in the right hemisphere. 9 animals survived and were sacrificed, under ether anesthesia, 8 days after surgery. Before the sacrifice a solution of Neutral Red (Koch Light Laboratories Ltd) was injected into the jugular vein for the subsequent assessment of the size of the ischemic areas. The colourless regions appeared to be ischemic on light-microscopic examination. After removal, the brains were observed in toto and then sectioned in 5 coronal slices. This method allowed us to

survey the presence of ischemic areas and their size. Particularly, the basal ganglia region appeared colourless in 2 animals, the cortical-subcortical region in 1 animal and the combined basal ganglia and cortical-subcortical regions in 5 animals. Lastly, in 1 rat there was no evidence of colourless areas (figure 2). No mortality occurred in 4 sham operated animals, and no colourless brain regions were observed.

The main limitations of this model lie in the need for general anesthesia and skull opening[16]. The advantage is the production of a focal cerebral ischemia without additional insults of hypoxia[1,11], hypotension[13,14] and handling of carotid and vertebral arteries[16]. Moreover, this model can be easily reproduced. Conversely, the model of transpalatine approach[7] involves a high rate of intra-operative accidents, mortality and postoperative complications consisting of wound infections and keratitis. The use of carbon microspheres for brain arterial embolization[10] involves multiple focal ischemic lesions. Furthermore, the permanent occlusion of many small vessels compromises the natural adjustments of the collateral cortico-pial circulation[18] and differentiates this model from the usual pathophysiological course of cerebral ischemia in man[19].

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The effect of cyclic AMP on dog renal function[1]

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Summary. The infusion of dibutyryl-cyclic AMP into the dog renal artery in vivo leads to diuresis, natriuresis and glucosuria. Addition of the nucleotide to the incubation medium bathing dog renal cortex slices in vitro causes inhibition of p-amino-hippurate accumulation and stimulation of glycine and β-methyl-glucoside transport. The results are interpreted in terms of the development of a blood-lumen flux of sodium and water in the renal proximal tubule, analogous to that seen in the intestine.

It is well established that a rise in the intracellular concentration of cyclic AMP in enterocytes is associated with an increase in the blood-lumen fluxes of sodium and chloride[2], though it is still unclear how the nucleotide exerts these effects. The direct influence of cyclic AMP on renal electrolyte transport mechanisms has not been examined in such detail; for that reason, we have studied the response of the dog kidney to an administration of the nucleotide via the renal artery using a recently described technique[3].

Methods. The experiments were performed on 4 mongrel dogs weighing around 30 kg. At the first intervention, the right kidney was removed and the left kidney was transplanted into the right iliac fossa, by means of end-to-end anastomoses to the iliac vessels[3]. 6–10 weeks later, the dog was re-anaesthetized, and the jugular vein was cannulated for the constant infusion of creatinine and p-amino-hippurate (PAH). After laparotomy, the ureter was cannulated, the sacral artery was ligated, and a catheter for the infusion of cyclic AMP was inserted[3]. For the 1st h, Krebs bicarbonate buffer alone was infused into the kidney at a rate of 0.5 ml/min; then dbcAMP (cyclic N^6,2′-O-dibutyryl-adenosine 3′:5′-monophosphate) was added to the infusion fluid at a dose level of 6 mg/kg · h and the infusion was continued for a 2nd h. Plasma and urine samples were taken every 15 min throughout the experiment for the determination of creatinine and PAH clearances, and sodium, potassium, chloride and glucose excretion rates.

At the end of the experiment, the kidney was excised for examination in vitro. Renal cortex slices of 0.4 mm thickness were incubated for 1 h in a ^{14}C-labelled solution of glycine, β-methyl-D-glucoside or p-amino-hippurate at a concentration of 0.1 mM and the uptake of these substrates into the tissues was determined as described previously[4].

The effect of an addition of dbcAMP to the incubation medium on the uptake of the above substrates was assessed in a separate series of experiments using cortex slices from normal dog kidneys. Incubations were also performed in a sodium-free, choline-substituted incubation medium in the presence and absence of dbcAMP.

Results. From the onset of the infusion of dbcAMP, there was a rise in the urine flow and a slight fall in the percentage reabsorption of sodium and chloride, but no consistent modification in the glomerular filtration rate. These changes were accompanied by a marked glucosuria (figure 1). When the perfused kidney was excised and studied in vitro, the uptakes of glycine, β-methyl-glucoside and PAH into cortical slices all fell within the normal range (results not shown).