from neurones intrinsic to the bowel, which is associated with an increased extrinsic, mainly adrenergic innervation.

VIP, substance P and PHI containing nerves[4,5,16] are major elements of the enteric nervous system and these results from the cat suggest that, in the anal sphincter, there is a reduced peptidergic innervation which is not seen in the other gut sphincters. The reduced level of intrinsic, including peptide-containing (fig. 2B), innervation in the internal anal sphincter provides a region of tonic contraction for retention of feces. This contraction is, presumably, overridden by nerves mediating the central nervous input and local autonomic reflexes to enable defecation.

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Aspects of interastrocytic gap junctions in blood-brain barrier in the experimental penumbra area, revealed by transmission electron microscopy and freeze-fracture

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Summary. Interastrocytic gap junctions in the blood-brain barrier of the experimental penumbra area were studied in the cat caudate nucleus 1 h after ischemia. Transmission electron microscopy and freeze-fracture studies revealed only slight changes in gap junctions between astrocytes, indicating that these junctions are very resistant to hypoxia.

The penumbra area[2] is a peri-infarct zone where the cerebral tissue can survive structurally but does not function electrophysiologically. These dormant neurons would benefit most from management strategies that increase blood flow in the area surrounding the infarct. In an experimental model of focal and selective cerebral ischemia[3], the penumbra area has been determined ultrastructurally after 1 h of ischemia (manuscript submitted for publication). Ultrastructural changes in the blood-brain barrier of the penumbra area, especially the endothelial cylinder, have been studied in different postischemic periods. Tissue functions depend on intact cellular membranes; an early response to an insult such as ischemia might well be reflected by molecular changes in membrane components[4]. Although many ultrastructural studies have described ischemic neurons there is no published information concerning molecular membrane structure in cerebral tissue after ischemia. The aim of the present study was to examine with transmission electron microscopy and freeze-fracture technique the gap junctions between astrocytic foot processes in the penumbra area after 1 h of circulatory arrest.

Material and methods. Experiments were performed in 5 cats weighing 2.5–3.5 kg, anesthetized with i.v. injection of pentobarbital (30 mg/kg) and 0.4 mg atropine. An endotracheal tube was installed, and a Harward respirator was used to maintain blood gases and pH within physiological limits. The anterolateral group of penetrating lenticulo-striated arteries arising from the M1 segment of middle cerebral artery and orbitofrontal artery origin were exposed through a transorbital approach and were occluded with bipolar coagulation using the lower current setting. The ischemic area produced was localized in the anterior part of the internal capsule and head of the caudate nucleus[3]. After 60 min of ischemia, the cats were fixed by retrograde perfusion through the abdominal aorta with a mixture of glutaraldehyde and paraformaldehyde[5]. After perfusion-fixation, the brain was left in situ for 1 h to eliminate dark cells, hydropic cells, and artifactitious perivascular spaces[6]. After dissection, samples of caudate nucleus were selected for transmission electron microscopy and the freeze-fracture technique. For electron microscopy, small tissue blocks of caudate nucleus were postfixed, after a buffer rinse, in 1% OsO_4 in 0.1 M sodium cacodylate buffer for 1 h. The tissues were then dehydrated in a graded series of ethanols, and embedded in Epon 812, with a transitional step in propylene oxide. 1-μm-thick toluidine blue stained sections from different caudate nucleus regions were selected for examination under the EM. Thin sections of silver-to-grey interference color were cut with a diamond knife mounted on a LKB ultratome. Sections were stained with uranyl acetate and lead ci-

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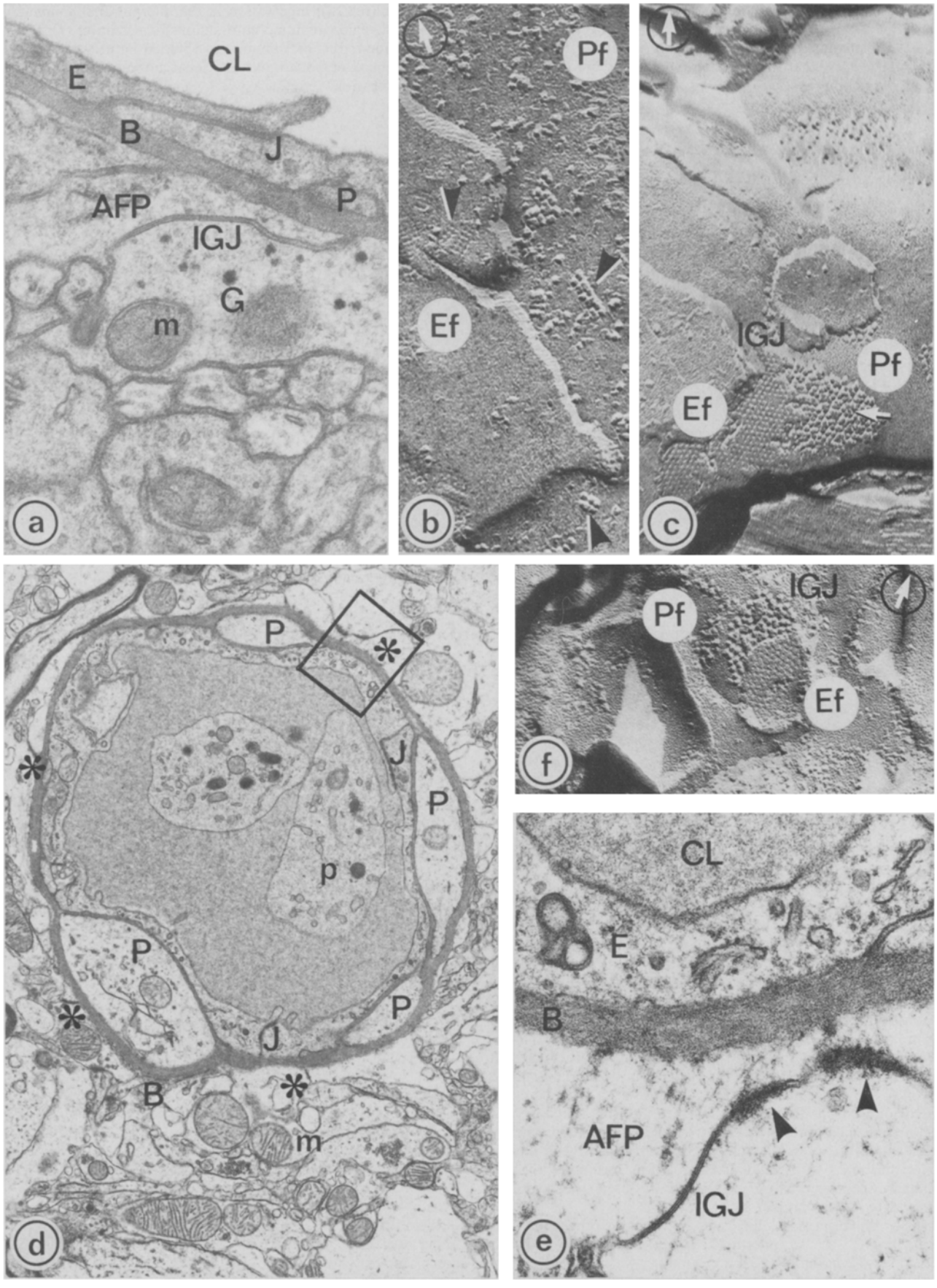

trate. For freeze-fracture, specimens were passed through a solution of 30% glycerol for 30 min and rapidly frozen in solid nitrogen at −196°C. Freeze-fracture, followed by etching for 60 sec, was performed in a Balzers BAF 400 unit. The etched fracture face was covered by platinum-carbon evaporated at a depth of 2–3 nm. The adherent tissue was removed from the replicas with Na-hypochlorite solution. The replicas were washed in distilled water and mounted on copper mesh girds. The thin sections and replicas were examined in a Philips EM 301. The non-ischemic contralateral caudate nucleus of the same animal was used as control. The nomenclature of Branton et al.[7] is used in this study.

Results and discussion. In control caudate nucleus, the blood-brain barrier is formed by a cylindrical capillary endothelium, pericapillary basal lamina and sheath of astrocytic foot processes. In some areas, the pericytic processes appear very close to the endothelium (fig. a). The astrocytic foot ends appear laterally joined by gap junctions and the cytoplasm contains glycogen in the form of β-particles (fig. a). After 60 min of experimental ischemia, the blood-brain barrier and inter-endothelial junction of the penumbra area preserve their anatomical integrity (fig. d) and endothelial organelles exist. The pericytic processes present slight signs of swelling, conserving their cytoplasmic structure (fig. d). The pericapillary basal lamina appears normal. Although a slight swelling can be observed in astrocytic foot processes, gap junctions are preserved between astrocytes (fig. d, asterisk). In some areas, the gap junctional area appears to be occupied by a highly electron dense material (fig. e, arrows). The mitochondria on astrocytic foot processes and surrounding neuropil are almost normal (fig. d).

In control animals, the membrane astrocyte studied with freeze-fracture presents aggregations of intramembrane particles called assemblies in P-face and complementary pit aggregations in E-face (fig. b). These assemblies form structures composed of 4–30 subunit particles (6.9–7.6 nm in diameter) arranged in an orthogonal pattern. The gap junctions between astrocytes of the control caudate nucleus show a crystalline disposition, with intramembrane particles in P-face and pits in E-face (fig. c).

In operated animals, gap junction size is smaller than in control animals; in order to obtain the diameter of gap junctions, the values of the long and short diameters were compiled as measured through the center of the junctions; the length and width were generally about 3.3 µm and 1.9 µm. In the penumbra area, the crystalline arrangement of gap junctions is more irregular and intramembrane particle density is lower in P-face (fig. f) than in control animals. In control animals, it was possible to observed pits in the center of particles of gap junctions (fig. c, white arrow). The pits in intramembrane particles of gap junctions represent the entrance to the ionic channel[8].

The characteristic features of astroglial cells observed in freeze-fracture replicas[9, 11] are as follows: a) orthogonal, small intramembrane particle (proteins) assemblies attached to the P-face (cytoplasmic leaflet of the membrane) with complementary orthogonal pit assemblies in the E-face (pits representing vacated attachment sites of P-face particles); and b) interastrocytic gap junctions in crystalline packing, most evident in E-face. The exact function of these protein aggregations is unclear. However, it is known that the astrocytic intramembrane particle assemblies disappear after oxygen deprivation[12]. In our experimental model of focal and selective cerebral ischemia, the astrocytic orthogonal assemblies disappear 10 min after circulatory arrest[11]. In addition, high levels of ATPase have been reported in astrocytic foot processes[13], a zone of high density of orthogonal assemblies[9]. It has been proposed that the biological function of glial gap junctions is directed to control ionic[14] and neurotransmitter[15] homeostasis during neuronal activity. The elevation of Ca^{2+} concentration is known to produce conformational change in each junctional particle which leads to an effective closure of the central channel[16] and it is interesting to note that cytosolic Ca^{2+} increases during reversible cerebral ischemia in the cat[17]. The different disposition of intramembrane particles (IMPs) observed in control (crystalline arrangement) and operated caudate nucleus may be related to conformational changes in the components of these particles. In order to substantiate this hypothesis, we are at present comparing the control and operated caudate nucleus to analyze the parameters of size, particle array and number of open ionic channels in gap junctions.

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a Blood-brain barrier in control cat caudate nucleus. CL, capillary lumen; E, endothelium; J, interendothelial junction; P, pericytic process; B, basal lamina. The astrocytic foot processes (AFP) are joined by a 7-layered gap junction. IGJ, interastrocytic gap junction; G, glycogen; m, mitochondria. × 38,500.

b The astrocytic plasmalemma is characterized by orthogonal particle assemblies in P-face (arrows) and by orthogonal assembled pits in the E-face (arrow). ×157,500.

c Crystalline disposition of particles (connexons) in the interastrocytic gap junction (IGJ) in control caudate nucleus. White arrow marks a pit in a particle of the P-face (Pf). Ef, E-face. ×129,000.

d Blood-brain barrier in penumbra area of caudate nucleus; p, platelets. The endothelial capillary cylinder appears joined by junctional complex (J). P, pericytic processes. Asterisk marks the interastrocytic gap junctions. B, basal lamina; m, mitochondria. ×11,250.

e Higher magnification of rectangle area of (d) CL, capillary lumen; E, endothelium; B, basal lamina; AFP, two astrocytic foot processes appear joined by a gap junction (IGJ). The 7-layered structure appears altered by an amorphous material in a portion of such junction (arrow). ×38,500.

f Freeze-fracture on a gap junction (IGJ) in the penumbra area; Pf, P-face; Ef, E-face. ×129,000.

In b, c, and f, the encircled arrow indicates the direction of shadow casting.