Brain capillary lesions produced by cocaine in rats

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Abstract

In the last 20 years, acute and chronic cocaine addiction has increased among young and adult people. The effects of cocaine on brain vasculature of young animals have not been histologically studied in depth. In the present study, we report the lesions of brain capillaries, including the choroid plexus, produced by chronic cocaine administration, in adult Wistar rats receiving i.p., 30 mg/kg/day of aqueous cocaine hydrochloride solution. Rats were sacrificed after several days of treatment. Histopathological examination of capillaries from different brain regions and cerebellum was performed using light microscopy. At 7 days, there were initial signs of dilatation, rupture and thrombosis of capillaries. At 15 days of treatment small interstitial oedema and hemorrhages by rupture of the basal membrane of the capillaries was found. At 30 days of treatment, many capillaries from different areas showed fibroid endothelial thickening, and wall fibrosis become evident after 60 days of daily cocaine. In numerous places (cortex, gray nucleus: thalamus, caudate, hippocampus and cerebellum) we observed capillaries with an occluded lumen probably due to fibrosis or thrombi after 90 days of treatment. In the latter treatment, capillaries from the choroid plexuses had their lumen dilated and the epithelial cells vacuolated or necrotic. We hypothesize that the chronic administration of cocaine in rats induced brain lesions in part as a result of capillary disruption and subsequent extravasation of erythrocytes to brain parenchyma. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

In the later stages of cocaine addiction people present neurologic and psychiatric complications (paranoid symptoms) (Barroso-Moguel et al.,

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1991) due to diffuse neuronal lesions secondary to the chronic cocaine abuse. Eplileptiform seizures or paralysis are also reported by Meyers and Earnest (1987), along with headache, delirium, transient loss of consciousness, agitation, anxiety or depression (Lowestein, 1987). Clinical and radiological examination of cases with brain and meningeal vasculitis of the main and medium size arteries have been reported (Lexa, 1995). Cocaine abuse is able to produce cerebral infarctions and extensive hemorrhages (Golbe and Merkin, 1986; Tuchman et al., 1987). It also induces an increase in arterial blood pressure, as well as frequent cardiac damage which may lead to infarctions or myocardial ischaemia (Coleman et al., 1982; Mathias, 1986), and cerebral vasculitis of small size vessels, (both meningeal and encephalic) (Lingeli and Bucheit, 1971; Morrow and Quillen, 1993). Since reports on the human and animal microscopic lesions produced by prolonged cocaine administration, on the blood vessels of the brain are scarce, we studied the evolution over the time of the lesions in capillaries of the brain of adult rats which chronically received cocaine injections.

In this work, we used light microscopy to describe the histopathological alterations of capillaries from several regions of brain after variable times or accumulative doses of exposure to cocaine.

2. Materials and methods

2.1. Reagents

Cocaine hydrochloride ($C_{17}H_{22}$ Cl NO_4) was obtained from the Department of Psychotropic Drugs from the Ministry of Health of Mexico. Formaldehyde and staining reagents were obtained from Mallinckrodt Baker (México) and all other reagents were from E. Merck (México).

2.2. Animals

We used 48 adult Wistar rats local strain (24 males and 24 females), of 40 days of age and with initial weight of 225–250 g. They were fed a standard chow diet (Purine Chow) and had free access to water. Room darkness was maintained between 19:00 and 07:00 h, room temperature at 25°C and relative humidity at 40%.

Twelve male and 12 female rats from the initial 48 were used as controls and received i.p., saline solution. The same number of rats received, i.p., 30 mg/kg/day of cocaine hydrochloride (equivalent to 26 mg/kg/day of free base cocaine) in saline aqueous solution for variable periods of time. This dose has been used in other reports to study long-term effects of cocaine in rats (Gordon et al., 1980; Barroso-Moguel et al., 1994). The above mentioned dose of cocaine did not produce mortality in any of the treatment groups. Groups of two rats from each treatment were sacrificed at different times: 7, 15, 30, 45, 60, and 90 days. Animals were anesthetized with 0.3 ml of 3.5% chloralhydrate i.p. and then perfused transcardially with saline solution, followed by 10% w/v formaldehyde aqueous solution at 4°C. Brains were dissected out and fixed in 10% formalin for during 15 days.Tissue samples were obtained from different brain areas. Tissue samples were paraffin-embedded and sectioned to $5-7\mu m$ thin slices. We applied hematoxylin-eosin, Masson's trichromic staining and Río-Hortega's argento-auric impregnation (Barroso-Moguel and Costero, 1962; Luna, 1960) techniques. Sections were then examined with a Zeiss light photomicroscope.

3. Results

No lesions in capillaries, perivascular spaces, brain parenchyma or cerebellum were observed in control rats (Fig. 1A).

From the start to 90 after i.p. daily cocaine injection, and 4 at 5 min after cocaine dosing, rats displayed a stereotyped behavior of the head and movements of anterior limbs during 30–45 min, followed of 2–3 h. of deep sleep. They ate and drank water normally.

We found no differences in cocaine-induced lesions between male and female rats. Therefore microphotographes were chosen independently of the rat gender.
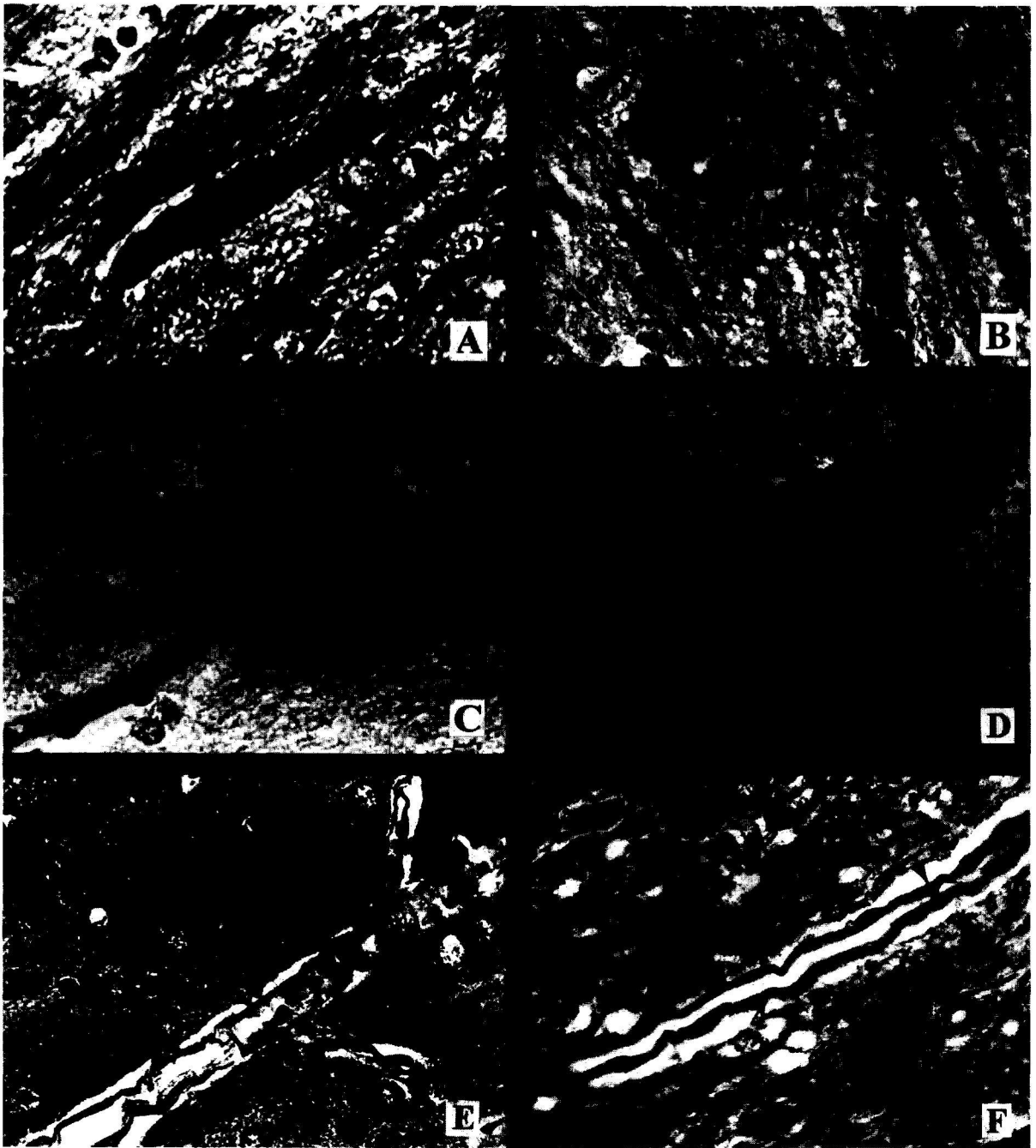
Fig. 1. Control rat with the normal appearence of capillaries in the globus pallidus (A), Occipital Cortex (C), Pyramidal layer in parietal cortex (E). At 7 days of cocaine treatment the capillaries present rupture, small thrombi and interstitial oedema in globus pallidus. (D) At day 15, capillaries with broken walls surrounded by erythocytes and macrophages (M) in occipital cortex. (F) At day 30 in parietal cortex, a capillar has an initial fibroid endothelial thickened with hyaline aspect (↑).

At day 7 of cocaine treatment, there was initial capillary dilatation in the *Globus Pallidus*, some of the brain capillaries showed rupture and small thrombi, interstitial hemorrhage and oedema (Fig. 1B). At day 15 of cocaine treatment there were alterations of the basal membranes of capillaries of the occipital cortex which appeared broken and surrounded by red corpuscles and macrophages. Interstitial oedema was evident and some neurons exhibited pyknotic nuclei (Fig. 1D). At 30 days of treatment, capillaries from parietal and frontal cortex, cerebellum and hippocampal areas of the brain showed initial fibroid endothelial thickened of their walls with hyaline aspect (arrows). There appeared to be few endothelial cells and pericapillary histiocytes, aside from an increase in the pericapillary space and interstitial oedema (Fig. 1F). At 60 days of treatment an intense hyaline thickening of the capillary walls decreased the lumen and the pericapillary space appeared augmented. The neurons (N) were scarce or were pyknotic, few were normal (*) and the oedema persisted (Fig. 2B). At 90 days of treatment the capillaries, in nucleus striatum and different areas of the brain, had completely occluded their lumen by fibrosis or thrombi. Some histiciocytes in the pericapillary space persisted. The interstitial oedema was more evident at 90 days. In most areas inflammatory cells or gliosis were observed (Fig. 2D).

The choroid plexuses after 90 days of cocaine treatment had important lesions in the epithelial cells of tortuous vessels. The rich capillary net showed an even bigger dilatation, with their epithelial cells in some parts preserved and in others altered. At this stage, there were not macrophages or others inflammatory cells present (Fig. 2F).

4. Discussion

With light microscope examination we found several capillary lesions, including interstitial hemorrhages, small thrombi, rupture of the brain capillaries and necrosis in choroid plexuses. Those destructive lesions appeared to have a direct vascular basis, as cocaine is a potent vasoconstrictor and vasoconstriction, in turn, frequently leads to a hypoxic response resulting in oedema and hemorrhage and subsequent tissue damage (Brent, 1990; Webster et al., 1991). Cocaine can also enhance blood clothing by stimulating platelets aggregation (Togna et al., 1985), which in turn can explain the presence of small thrombi found in our study.

Some authors (e.g., Silva-Araujo et al., 1994; Méndez-Armenta et al., 1997) found similar oedema and hemorrhagic lesions and ischemia/necrosis in retina of neonatal rats exposed to cocaine. In previous studies with rats, we showed testicular and kidney lesions by the action of cocaine that could also be of vascular origin (Barroso-Moguel et al., 1994, 1995). Cocaine blocks the reuptake of norepinephrine at nerve endings resulting in an increased circulating level of catecholamines (Fleming et al., 1990), which, in turn, produced vasoconstriction, oedema, necrosis of the seminiferous tubules and glomerular and tubular lesions.

In human studies, Kaye and Fainstat (1987) and Rumbaugh et al. (1971) reported, in an angiographic vasculitis study of the human nervous system associated with cocaine abuse, an involvement of small arterioles and capillary segments of cerebral vessels. In those *post-mortem* human studies they described diffuse oedema, ischemic cell injury and hemorrhagic lesions around small sized vessels. Krendel et al. (1990) reported a pathological description of vasculitis associated with cocaine abuse by cerebral biopsy, three weeks after the onset of neurologic symptoms produced by smoking of crack cocaine, revealing acute inflammatory cells infiltrating the walls of small cortical vessels and an area of necrosis coincident with infarction.

On the other hand, the cocaine metabolite benzoilecgonine (BE) is present in high concentrations in the plasma within 30–60 min. after injection of cocaine in humans (Fleming et al., 1990). In adult animals, BE has been shown to have a potent stimulatory effect on nerve cells which may be in part related to the ability of this active metabolite to form a molecular complex with calcium ions with subsequent alteration of all processes in neurons and endothelial cells of capillaries which are regulated by calcium ion (Spear et al., 1989).
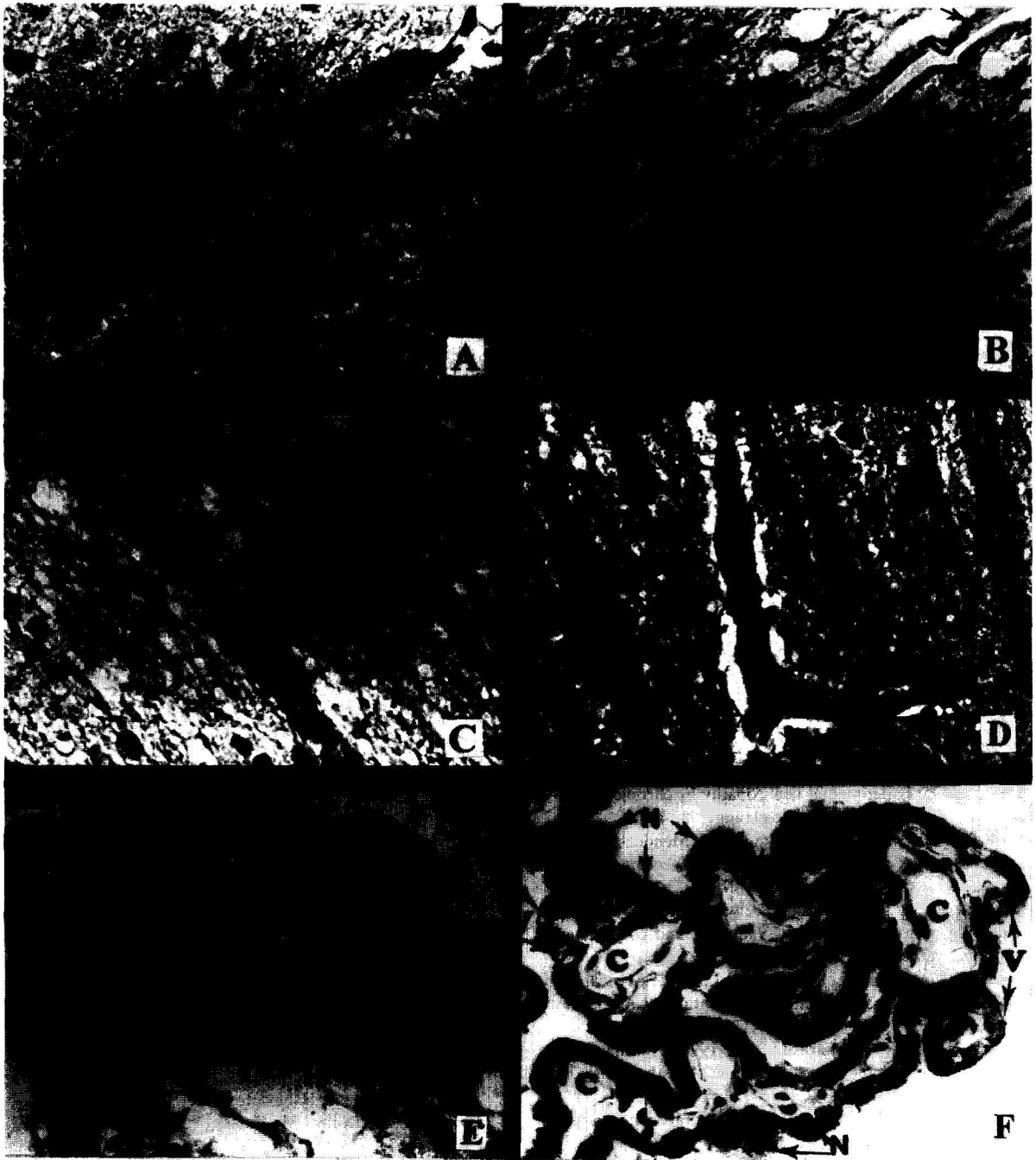
Fig. 2. Control rats with normal appearance of capillaries in the caudate nucleus (A), Striated nucleus (C) and choroid plexuses (E). (B) At day 60 of cocaine treatment (B, D, F) there is a decrease of capillary lumen in the caudate nucleus. (D) At day 90th, one of the various capillaries in striated nucleus with lumen completely occluded by fibrosis. Rio-Hortega's stain 100 × . (F) Choroid plexuses at 90 days of cocaine treatment showing dilated capillaries (C). In some places the epithelial cells seems to be vacuolated (V), or necrotic (N). There are no inflammatory cells. Masson's method 100 × .

In addition to the dopaminergic effects of cocaine, an NMDA-receptor involvement in the mechanism of action of cocaine has been claimed to explain mortality and seizures that accompany neurotoxicity of the drug (Shimosato et al., 1995). As brain vessels also express NMDA receptor (Koenig et al., 1992; Stastny et al., 1997), it is possible that cocaine mediated action on this receptor can also be involved in the damage observed in the present study.

In summary, this study showed that long-term cocaine exposure to rats produced severe damage in brain capillaries, notably oedema, hemorrhages and necrosis in the cerebral parenchyma and choroid plexuses, suggesting that the capillary lesions are secondary to vasoconstrictor effect of cocaine, as similar lesions have been found in human adults and neonates exposed to cocaine.

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