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Blood-Brain Barrier Dysfunction after Amphetamine Administration in Rats

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Summary. Amphetamine administration to rats anaesthetized with nitrous oxide resulted in protein extravasation in the brain, particularly in the frontoparietal cortex. The drug is known to increase the cerebral blood flow in the same areas, indicating a vasodilatory action on the cerebral vessels. Protein leakage could be prevented by lowering the blood pressure and, to a large extent, also by hyperventilation. This suggests that the permeability disturbance is caused by the mechanical stress which results from high intraluminal pressure in combination with vasodilatation.

The pharmacological effect of amphetamine is caused by a catecholamine release in the central nervous system and in the periphery. The vasodilatory effect of amphetamine could be either a direct effect on the cerebral vessels or could be secondary to an increased cerebral metabolism. The vasodilatory beta-adrenergic receptors in the cerebral vessels are of $beta_1$ type. Practolol, a selective $beta_1$-receptor antagonist which can prevent the vasodilatory effect of isoprenaline on cerebral vessels, did not influence the amphetamine induced protein leakage. In contrast, propranolol, a non-selective beta-receptor blocking drug which earlier has been shown to prevent catecholamine induced increase of CBF and oxygen consumption, diminished or prevented the protein leakage. It is likely that the cerebral vasodilatation caused by amphetamine, at least in part, is secondary to an increased cerebral metabolism induced by a catecholamine release.

Key words: Blood-brain barrier – Amphetamine – Blood pressure – Cerebral blood flow – Protein tracers – Hyperventilation – Propranolol – Haloperidol.

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Administration of large doses of amphetamine sulphate to rats under nitrous oxide anaesthesia increases the cerebral blood flow (CBF) and oxygen consumption, (Carlsson et al., 1975; Berntman et al., 1978). The increase in CBF is of a similar magnitude as that seen in rats during hypercapnia (Eklöf et al.) and epileptic brain activity (Meldrum and Nilsson, 1976; Chapman et al., 1977) indicating a high degree of vasodilatation. Recent studies have demonstrated that the blood-brain barrier (BBB) dysfunction occurring in experimental epilepsy and in hypercapnia is pressure dependent (Bolwig et al., 1977; Johansson and Nilsson, 1977; Petito et al., 1977), suggesting that the arterial blood pressure is a decisive factor for the development of BBB dysfunction in these conditions when the resistance vessels are maximally dilated.

In the experimental models with high CBF mentioned above a mean arterial blood pressure (MABP) exceeding 150 mm Hg is usually associated with BBB dysfunction (Johansson and Nilsson, 1977). The MABP in rats anaesthetized with nitrous oxide is in the range of 135–155 mm Hg, i.e. higher than in conscious rats or in rats under barbiturate anaesthesia. As the MABP increases and the resistance vessels dilate after amphetamine administration, it could be expected that this drug given to rats under nitrous oxide anaesthesia would result in a disturbance of the BBB. Preliminary experiments showed that protein leakage did occur both after i.p. and i.v. administration of the drug. In the present experiments the extent and location of protein extravasation in the rat brain was studied after amphetamine administration under various experimental conditions that theoretically could influence the permeability. In particular, the effect of some receptor antagonists thought to be of importance for the effect of amphetamine on the brain and the cerebral blood vessels, was studied.

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Methods

Male Sprague Dawley rats, with free access to rat pellets and to water, were anaesthetized with divinyl ether, trachetomized, and immobilized with i.v. tubocurarine chloride (0.5 mg · kg^{-1}). Thereafter the animals were ventilated with 70% N_2O and 30% O_2 with a small animal ventilator. One femoral artery was cannulated for blood pressure recording and anaerobic sampling of blood for blood gas analyses and one femoral vein for injection of drugs and tracers.

The rats received *dl*-amphetamine sulphate in a dose of 2.5 mg · kg^{-1} i.v. To test the blood-brain barrier function Evans blue (EB), 3 ml · kg^{-1} of a 2% solution in saline, was given i.v. before amphetamine administration. With this dose, the tracer binds to serum albumin and the dye-protein complex (EBA) can be traced by fluorescence microscopy (Steinwall and Klatzo, 1966). In addition, ^{125}I-labelled human serum albumin (^{125}IHSA) was given to some groups (see Tables 1 and 2).

Experimental Groups (the number of animals in each group is given in Table 1). Controls were given EB and ^{125}IHSA only. Normo- and hypocapnic rats received amphetamine and no other drug; one group of each was used for careful evaluation of EBA extravasation, the other for quantitative determination of protein leakage. In another experimental group the MABP was lowered by bleeding. In the remaining groups one of the following receptor antagonists were given 30 min (haloperidol 60 min) before amphetamine: haloperidol, a dopamine and noradrenaline receptor blocking drug (1 mg · kg^{-1}): pimozide, a selective dopamine receptor antagonist (1 mg · kg^{-1}): propranolol—a non-selective beta-receptor antagonist (1 mg and 3 mg · kg^{-1}): practolol—a selective $beta_1$-antagonist (3 mg · kg^{-1}).

Thirty minutes after the tracers were given in the controls and 30 min after amphetamine administration in the other groups, the animals were killed by bleeding and the brain perfused with saline for 30 s to rinse the blood out of the vessels and, unless ^{125}IHSA had been given, the brains were further perfused with a formaldehyde solution. The brains were photographed, and the location of EBA extravasation registered and sections taken for fluorescence microscopy (for details, see Johansson et al., 1970). Brains containing ^{125}IHSA were subjected to routine scintillation counting. The albumin content was expressed as 100 (counts · min^{-1} · mg^{-1} brain tissue)/(counts · min^{-1} · mg^{-1} blood). The blood samples were taken immediately before killing the animals.

Results

The Pa_{O_2} was $\geqq$ 100 mm Hg (13.3 k *Pa*) in all animals. Table 1 shows the MABP before and after amphetamine administration in the different groups, as well as Pa_{CO_2} values and degree of EBA extravasation. The brains from the 12 normocapnic rats given amphetamine all showed EBA extravasation in the brains. Hyperventilation decreased the BBB dysfunction. Lowering the MABP before amphetamine administration prevented protein leakage and so did pretreatment with haloperidol, which also abolished the blood pressure increase. Propranolol diminished the BBB dysfunction without preventing the pressure increase.

Table 1. Maximal MABP before and after *dl*-amphetamine (2.5 mg · kg^{-1} i.v.) and Pa_{CO_2} at the time of drug injection (M ± S.E.M.). Visual extimation of EBA extravasation in the brain. For definition of grade 0—4, see the text

Groups	Tracers	*n*	MABP before amphetamine mm Hg	MABP after amphetamine mm Hg	Pa_{CO_2} *k Pa*	EBA extravasation (grade 0—4)				
						0	1	2	3	4
Controls (no amphetamine)	EB, ^{125}IHSA	6	149 ± 2	–	5.0 ± 0.11	6	0	0	0	0
Normocapnia	EB	6	147 ± 2	173 ± 7	5.0 ± 0.29	0	1	1	2	2
Normocapnia	EB, ^{125}IHSA	6	152 ± 5	183 ± 6	5.0 ± 0.09	0	1	1	3	1
Hypocapnia	EB	6	159 ± 2	192 ± 4	3.3 ± 0.14	2	3	0	1	0
Hypocapnia	EB, ^{125}ISHA	6	158 ± 3	204 ± 8	3.4 ± 0.13	4	2	0	0	0
MABP lowered by bleeding	EB	4	107 ± 6	125 ± 3	4.6 ± 0.03	4	0	0	0	0
Haloperidol (1 mg · kg^{-1})	EB	6	132 ± 5	123 ± 7	5.0 ± 0.19	6	0	0	0	0
Propranolol (1 mg · kg^{-1})	EB, ^{125}IHSA	4	153 ± 5	188 ± 12	5.3 ± 0.26	2	1	1	0	0
Propranolol (3 mg · kg^{-1})	EB, ^{125}IHSA	4	146 ± 4	181 ± 2	5.1 ± 0.16	4	0	0	0	0
Practolol (3 mg · kg^{-1})	EB	6	148 ± 3	198 ± 3	4.9 ± 0.11	0	1	2	2	1
Pimozide (1 mg · kg^{-1})	EB	6	142 ± 4	190 ± 4	4.8 ± 0.12	0	2	0	2	2

Table 2. ^{125}IHSA uptake in rat brain after amphetamine administration expressed as 100 (counts · min^{-1} · mg^{-1} brain tissue)/(counts · min^{-1} · mg^{-1} blood) M ± S.E.M.

Groups	*n*	Frontal cortex	(Range)	Diencephalon and mesencephalon	Pons and medulla oblongata	Cerebellum
Normocapnia	6	2.22 ± 1.32	(0.24—8.53)	0.15 ± 0.05	0.09 ± 0.02	0.09 ± 0.02
Hypocapnia	6	0.05 ± 0.01	(0.04—0.10)	0.05 ± 0.004	0.08 ± 0.02	0.06 ± 0.01
Propranolol 1 mg · kg^{-1}	4	0.13 ± 0.06	(0.05—0.30)	N.D.	N.D.	N.D.
Propranolol 3 mg · kg^{-1}	4	0.03 ± 0.004	(0.03—0.05)	N.D.	N.D.	N.D.
No amphetamine	6	0.03 ± 0.003	(0.02—0.04)	N.D.	N.D.	0.03 ± 0.003

N.D. = not done

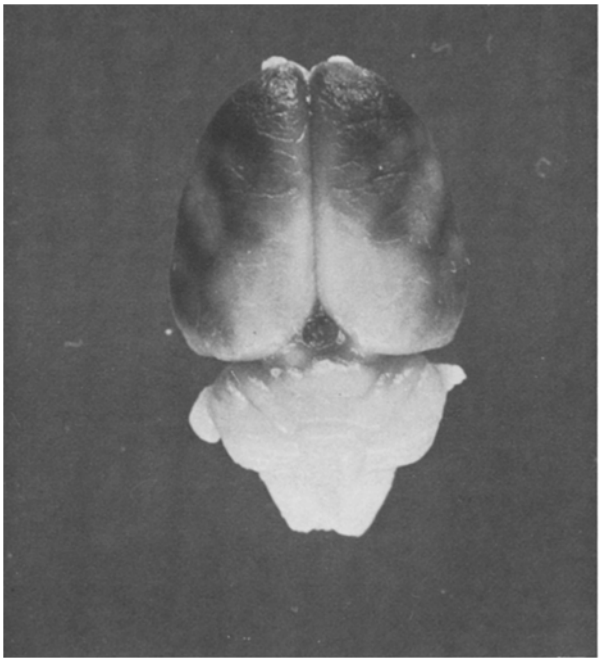

Fig. 1. Evans blue-albumin extravasation (dark areas) in the cerebral cortex in a rat brain after *dl*-amphetamine administration. The degree of protein extravasation corresponds to grade 3 in Table 1 and the text

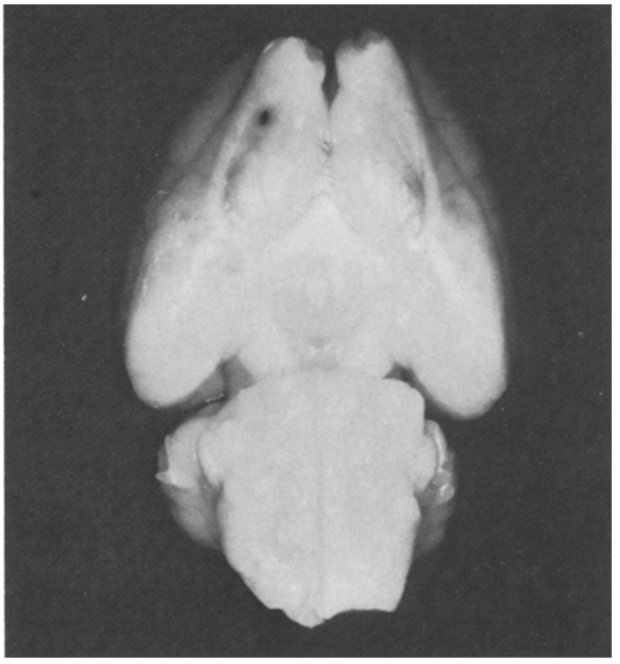

Fig. 2. A small hemorrhage medial to the right olfactory tract is seen in the brain of a rat killed 30 min after i.v. administration of *dl*-amphetamine (2.5 mg · kg^{-1})

Pimozide and practolol did not influence the protein leakage.

On the *convex surface* of the brain areas of protein extravasation were predominantly seen in the frontal and parietal lobes. The range of extravasation could roughly be described with the following grades. Grade 0 = no extravasation; grade 1 = mainly extravasation in the anterior and medial part of the frontal lobes; grade 2 = extensive extravasation in frontal lobes and lateral parts of the parietal lobes; grade 3 = in addition to extensive extravasation in the frontal and parietal lobes, lateral areas of the occipital and temporal lobes were affected; grade 4 = extravasation occurred over the whole convexity except the most medial part of the occipital lobes. A typical example of tracer extravasation, grade 3, is seen in Figure 1.

On the *basal surface* of the brains the olfactory tubercules and tracts were most affected. The posterior part of the pyriform lobes was usually not stained.

On *coronal sections* the tracer extravasation was mainly confined to cortical areas, but occurred also in the basal ganglia, particulaly in the caudate nucleus, in rats with more extensive BBB dysfunction. EBA extravasation was rarely seen in the diencephalon, the mesencephalon, the pons, the medulla oblongata or the cerebellum. Thus, of all the brains invesigated a few small spots of EBA extravasation were seen in any of these regions in only four animals. However, the experiments with ^{125}IHSA indicate that a slight protein leakage might occur also in these parts of the brain.

More than one third of the brains with BBB dysfunction showed at least one pinpoint size hemorrhage, most commonly on the basal surface medial to the olfactory tract (Fig. 2). In two brains such hemorrhages were found in the frontal cortex and in one of these brains also in the parieto-occipital region.

In fluorescence microscopy the tracer EBA was seen in the vessel walls, both in pial arteries, intracerebral arterioles and smaller vessels (capillaries and venules). Extravasation of the tracer was noted both as a diffused fluorescence of the neuropil and as fluorescent cell bodies, particularly neurons but occasionally also glia cells. There was a good correspondence between macroscopic EBA extravasation and the extravasation seen in fluorescence macroscopy but in a few brains some arterioles contained the fluorescent tracer in cortical areas that were macroscopically negative.

^{125}IHSA content in various parts of the brain in the groups given this tracer is presented in Table 2.

Discussion

The BBB dysfunction induced by amphetamine can be prevented by lowering the blood pressure and, to a

large extent, also by hyperventilation; thus it is unlikely that amphetamine per se increases the permeability. The excessive increase in CBF reported by Carlsson et al. (1975) and Berntman et al. (1978) indicates a marked degree of vasodilatation. Since the vessel wall tension is a function of the intraluminal pressure and the radius, dilated vessels are exposed to considerable stress when the pressure is high (cf. Johansson and Nilsson, 1977). The preventive effect of hyperventilation illustrates that vasoconstriction decreases the mechanical stress as earlier shown in acute hypertension (Johansson, 1976a, b).

The pharmacological effect of amphetamine is due to a release of catecholamines in the periphery and in the central nervous system (Stein, 1964; Andén, 1970; Carlsson, 1970; Sulser and Sanders-Buch, 1970). Theoretically the vasodilatory effect of amphetamine could be a direct effect on the cerebral vessels or it could be secondary to an increased cerebral metabolism. An autoradiographic study on the distribution of ^{14}C amphetamine in the mouse brain showed that the radioactivity accumulated preferentially in the cerebral cortex (Benakis and Thomasset, 1970). A recent study on regional cerebral blood flow in rats after amphetamine administration showed that the flow increased predominantly in frontoparietal cortical areas (Berntman et al., 1978) i.e. regions showing extensive protein leakage in the present study. In the cerebral vessels the highest number of adrenergic nerve terminals are found in the anterior and middle cerebral arteries and the vasodilatory beta-adrenergic receptors are of the $beta_1$ type (Edvinsson and Owman, 1974). The selective $beta_1$-receptor antagonist practolol which prevents the cerebral vasodilatation induced by isoprenaline, a potent $beta_1$ agonist (Sercombe et al., 1977), had no effect on the protein leakage in our experiments. There is also some evidence that the cerebral vessels contain vasodilatory dopamine receptors which can be blocked by haloperidol or pimozide (von Essen, 1974). Although haloperidol prevented protein leakage in our experiments this was more likely due to the lowering of the blood pressure. Pimozide, a more selective dopamine receptor antagonist had no such effect. Moreover, amphetamine has no vasodilatory effect on cerebral vessels in vitro (J.-E. Hardebo, personal communication). We have thus no evidence that the cerebral vasodilatation induced by amphetamine is a direct effect on the cerebral vessels.

Propranolol does not per se change the CBF in rats (Carlsson et al., 1976), but has been found to prevent a catecholamine induced increase in CBF and oxygen consumption in immobilization stress (Carlsson et al., 1977). When exogenous norepinephrine gains access to the brain or when endogenous norepinephrine is released pharmacologically a rise in CBF and metabolism results and this effect can be blocked by propanolol (MacKenzie et al., 1976a, b). The fact that the drug decreased the protein extravasation in the present experiments-without affecting the blood pressure increase – is consistent with the hypothesis that the vasodilatory effect of amphetamine on the cerebral vessels may be secondary to an increased metabolism. *dl*-propanolol has in addition to the receptor blocking effect a membrane-stabilizing (i.e. local anaesthetic effect) which theoretically could influence the permeability. However, *d*-propranolol, which has the same membrane-stabilizing effect but very little effect on the beta receptors does not prevent amphetamine induced protein leakage (unpublished observations).

In conclusion, it seems likely that the vasodilatory effect of amphetamine on cerebral vessels, at least in part, is secondary to a catecholamine induced increase in cerebral metabolism, as also suggested by McCulloch and Harper (1977).

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