Changes in blood-brain permeability resulting from d-amphetamine, 6-hydroxydopamine and pimozide measured by a new technique[1]

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Summary. A new technique is described for measurement of diffusion across the blood-brain barrier using intraventricularly administered ^{68}Ga-EDTA, and determining loss from the brain with a scintillation camera. Repeated injections via permanent cannulas showed that the diffusion half-time was reduced to 50% of control values after intraventricular d-amphetamine and 6-hydroxydopamine; pimozide had no effect.

Although the blood-brain barrier (BBB) has not yet been satisfactorily defined either in anatomical or in biochemical terms, it seems to function at the level of the capillary wall between the lumen of the blood vessels and the brain tissue. Generally, lipid-soluble molecules pass through the plasma membrane readily; passage of ions, proteins and large lipid-insoluble molecules occurs at tight junctions and the lateral intercellular space, while water and small non-electrolytes seem to pass through the cells via the plasma membrane. The BBB enables a selective passage of substances from the circulating blood into the brain, thus protecting the brain from variations in blood composition and from the entry of toxic compounds. One of the current theories as to the metabolic basis of schizophrenia postulates a hyperdopaminergic condition. This could result from a partial or selective failure of the BBB admitting excessive amounts of dopa, normally excluded by the action of aromatic amino acid decarboxylase (AADC) in the brain capillary walls. Excessive dopa could also result in psychotoxic metabolites[3], which might conceivably alter the BBB.

We report here a new method for measuring the rate of diffusion out of the brain, utilizing intraventricular administration of ^{68}Ga-EDTA. Although this is not a physiologically normal material, it has a mol wt of 370 comparable to non-electrolyte physiological compounds, and its form is not altered by metabolism. Preliminary studies with non-chelated ^{99m}Tc and ^{68}Ga showed that these materials remained in the brain and did not diffuse out, and hence were of little value for this study. ^{68}Ga-EDTA has been used successfully as a brain-scanning agent in nuclear medicine[4-6], where it is administered i.v. Pathology which results in failure of the BBB allows accumulation of ^{68}Ga-EDTA at the site of the pathology which is visualized with a scintillation camera. In our method, the outward diffusion of intraventricularly administered ^{68}Ga-EDTA is measured by the rate of its disappearance from the brain.

^{68}Ga-EDTA does not deposit in the bone or calvarium and does not deposit in organs other than kidneys and bladder. The initial disappearance half-time from the blood is approximately 10 min, and the rate of passage through the kidneys to the bladder is quite rapid, even in the dehydrated animal[7]. ^{68}Ga is a convenient isotope because it has a very short half-life (68.3 min), allowing repeat studies at short intervals; emits positrons which are converted in tissue into 2 coincident 0.51 MV gamma rays 180° apart; and may be conveniently prepared by milking the parent ^{68}Ge isotope ($T_{1/2}$=275 d) which is fixed on an ion exchange column[8]. By selecting the highest concentration fraction of eluate from the column it is possible to obtain sufficient radioactivity in a volume of 1.5 λ, suitable for intraventricular injection. We chose a small group of test compounds for our initial study because of their relationship to various aspects of dopamine metabolism. 1. d-Amphetamine in man after chronic increasing use produces a syndrome indistinguishable from paranoid schizophrenia.

$T_{1/2}$'s and zero-time intercepts of the long half-time component of diffusion of ^{68}Ga-EDTA from brain of rats after pre-treatment with various drugs. p-values are from 't' statistic evaluation for paired observations, 2-tailed test

Treat drug and dosage	Rat number	Intercept (%)	$T_{1/2}$ (min)	$T_{1/2}$ (% of control)
Control	1	89	146	100
	2	87	184	100
	3	90	248	100
	Mean	89	193	
Amphetamine (100 µg in 1 µl)	1	80	64	44
	2	93	87	47
	3	80	134	54
	Mean	84	95	48
	p	n.s.	<0.05	
Pimozide (0.12 µg in 1 µl)	1	82	131	90
	2	87	193	105
	3	92	268	108
	Mean	87	197	101
	p	n.s.	n.s.	
6-OHDA (200 µg in 2 µl)	1	83	60	41
	2	74	78	42
	3	81	102	41
	Mean	79	80	42
	p	n.s.	<0.025	
6-OHDA (30 days later)	1	86	146	100
	2	93	180	98
	3	91	252	102
	Mean	90	193	100
	p	n.s.	n.s.	

n.s., not significant.

In animals it causes an excessive release of dopamine from storage granules and blocks re-uptake from the synaptic cleft, which presumably is also responsible for the clinical effect in man. Many ring-methoxylated congeners of amphetamine produce psychotomimetic effects in man with a single administration[3]. 2. 6-Hydroxydopamine (6-OHDA) produces blockade and eventual degeneration of noradrenergic and dopaminergic neural tracts. It has been postulated that if it were produced endogenously and methoxylated it would provide a psychotoxin which might be the basis of schizophrenia[3]. Stein and Wise subsequently proposed that 6-OHDA itself is the psychotoxin[9], by virtue of the damage it can produce in the reward system of the brain. 3. Pimozide, a recently developed neuroleptic, has been shown in animal studies to specifically block dopamine receptors in the brain, with little or none of the blocking of norepinephrine receptors shown by previous neuroleptics[10].

Methods. Permanent guide cannulas were implanted into the ventricles of 3 male Sprague-Dawley rats, using a Kopff stereotactic apparatus, and cemented to the skull and an anchor screw with dental cement. After a 10-day recovery period, the animals (weighing 250–300 g) were anesthetized with ca 0.3 ml Diabutal i.p. to maintain immobility for 120 min while stretched on a board. ^{68}Ga-EDTA was eluted carrier-free with 0.005 M EDTA from an alumina column containing the ^{68}Ge parent[8], sterilized by millipore filtration, and adjusted to physiological or slightly hypertonic solution at a concentration of 20–60 mCi/ml. 1–2 µl was injected with a Hamilton µl-syringe into one of the guide cannulas, yielding a count rate of ca 20,000 cpm at the time of injection.

The animal was placed immediately under the Anger positron scintillation camera. This device, built by Anger at this laboratory[4], uses the coincidence of the two 0.51 MeV gamma rays from the positron conversion process to collimate and image the radioactive source with a spatial resolution of 0.7 cm. The animal was counted alternately in 2 geometries for 2-min intervals: a) with the head only in the field of view; b) with the whole body in the field of view. Scintiphotos of the whole body were taken periodically to visually determine the location of the isotope. The counts from the head only were corrected for decay and expressed as a percent of the first count; the whole body views were to establish organ localization and net balance of counts.

Each animal was its own control; after determination of the diffusion of ^{68}Ga-EDTA out of the brain without pretreatment, 3 or more days later one of the test compounds was injected intraventricularly in a volume of 1–2 µl. The amount of pimozide injected was limited by its solubility. 30 min later ^{68}Ga-EDTA was again administered and the rate of disappearance from the brain measured. After a period of at least 1 week, the next test compound was administered. 30 days after 6-OHDA was tested, ^{68}Ga-EDTA was again administered to determine whether the diffusion had returned to control values.

Results. Data obtained from a typical animal which received ^{68}Ga-EDTA intraventricularly after the various pretreatment drugs is shown in figure 1. The amount of ^{68}Ga (decay corrected) retained in the brain plotted on a logarithmic scale as a function of time yields curves which can be described by the sum of 2 exponential functions. The principal exponential has a half-time of 146 min for this animal, with an intercept of 89%. The fast component has an intercept of 11% and a half-time of 2–3 min. The half-times and intercepts of the principal components for each animal and each pretreatment drug are shown in the table. The control half-times ($T_{1/2}$ min, table) were quite variable, but letting each animal serve as its own control results in percentage changes ($T_{1/2}$, percent of control, table) which are tightly grouped.

As can be seen in the table, pretreatment with d-amphetamine increased the rate of diffusion of ^{68}Ga-EDTA out of

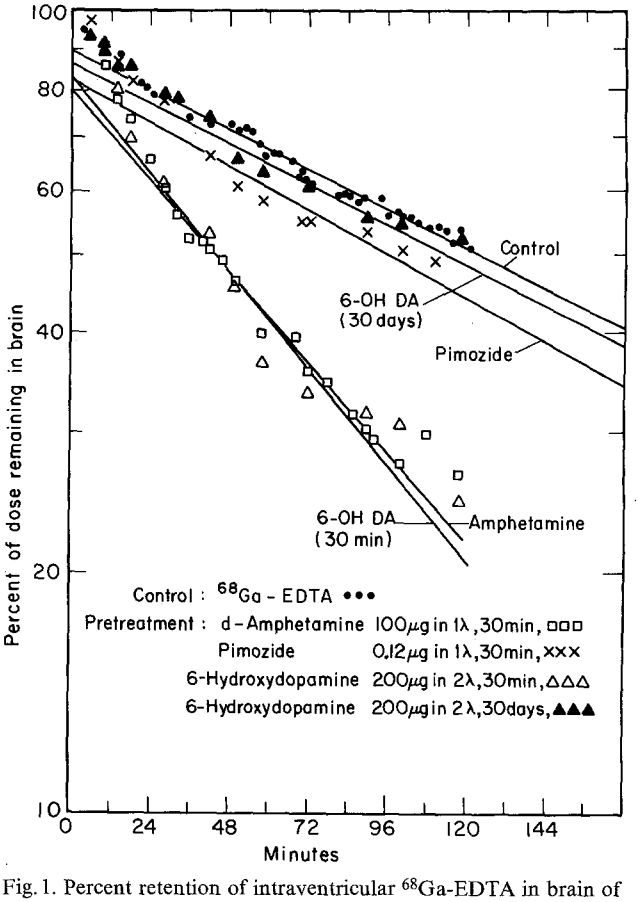

Fig. 1. Percent retention of intraventricular ^{68}Ga-EDTA in brain of rat No. 1 as a function of time after injection. Indicated drugs were administered intraventricularly 30 min prior to ^{68}Ga-EDTA, for comparison to control with no pre-treatment.

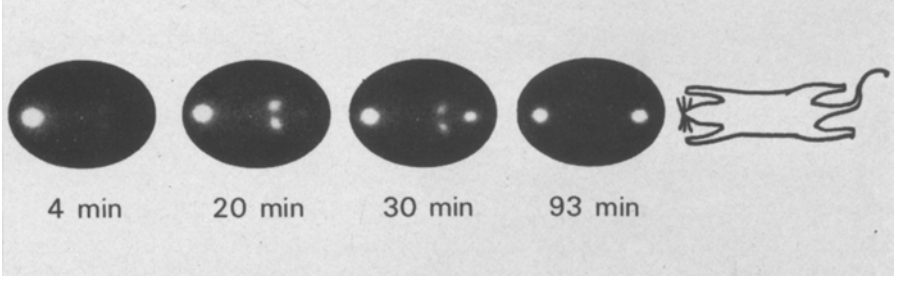

Fig. 2. Scintiphotos of entire rat at various times after intraventricular injection of ^{68}Ga-EDTA, as seen with Anger camera in positron mode.

the brain, reducing the half-time from a mean of 193 min to 95 min, 48% of the control value ($p < 0.05$). 6-OHDA had a comparable effect, reducing the half-time to 80 min, 42% of control ($p < 0.025$). 30 days after 6-OHDA treatment the diffusion half-time had returned to control values. The mean diffusion half-time after pimozide pretreatment was 197 min, not significantly different from controls. The intercepts and half-times of the fast component did not appear to be significantly altered by any of the pretreatment drugs. Representative scintiphotos in figure 2 show that the ^{68}Ga was initially in the brain, then appeared transiently in the kidneys and then began to concentrate in the bladder; it was not seen in any other organs.

Discussion. The results obtained here are quite similar to those found for diffusion of ^{24}Na from blood into CSF by Levin and Patlak[11]. These authors found a rapid component with a $T_{1/2}$ of about 5 min and intercept of 0.56, and a 2nd, slower component of $T_{1/2}$ about 2 h; with some reservations, they attributed these to blood-CSF exchange and brain extra-cellular space (ECS) - CSF exchange, respectively. The fact that the intercept for the fast component found here was smaller, 0.11, probably reflects the fact that the label was placed in the CSF rather than in the blood, allowing a proportionately greater fraction to enter the ECS of the brain.

It is difficult to compare the results of the many different methods of measuring brain diffusion parameters, and we have not developed a mathematical model of analysis of our data. All authors seem to agree, however, that diffusion half-times are valid indicators of the status of the BBB with respect to the molecule being used as an indicator. The relative simplicity of our method compared to other techniques allows comparison of the effects of various drugs on the brain diffusion of a non-electrolyte which labels the ECS. It allows comparison of the effects of drugs in the same animal, an important consideration when the control values are as variable among animals as found here, yet they are consistent on repeat tests in the same animal.

In these initial studies we have investigated the effects of 2 drugs which have been linked to schizophrenia. Both amphetamine and 6-OHDA increased the diffusion of ^{68}Ga-EDTA out of the brain, reducing the $T_{1/2}$ by a factor of 2. Pimozide, which is believed to exert its anti-psychotic action by blocking the post-synaptic DA receptors, had no effect. It seems too simplistic to suggest that amphetamine, in addition to its known effects, induces psychotic symptoms by simply lowering the brain's barrier to toxins, or specifically to dopa which would be immediately converted to dopamine thus producing a hyperdopaminergic state. Although there is little or no evidence that 6-OHDA occurs in brain, it has the same effect on the BBB in our experiments as amphetamine. Although 6-OHDA is known to produce irreversible damage to nonadrenergic and dopaminergic tracts in the CNS, the effect on the BBB had disappeared by 30 days. It can only be assumed in these studies that passive diffusion as measured with ^{68}Ga-EDTA is the same in both directions across the BBB.

The method described here is potentially applicable to any compounds which can be labelled with a gamma-emitting radioisotope of energy suitable for use with a scintillation camera, i.e., with gamma energies less than $\simeq 400$ keV, or which emits positrons. Ideally, the compound should also not be appreciably metabolized and be selectively removed by the kidney. The method should be applicable to the study of a variety of other compounds which could be appropriately labelled, and the effects of various drugs on their diffusion out of the brain.

1 Acknowledgment. This work was supported by the Division of Biomedical and Environmental Research of the US Department of Energy, and the Deutsche Forschungsgemeinschaft.
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Antidiuretic and thermogenic effects of intracerebroventricular prostaglandin H_2 in ethanol-anaesthetized rats

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Summary. When PGH_2 was administered intracerebroventricularly at doses of 5 and 15 nmoles in ethanol-anaesthetized rats, alcohol diuresis was inhibited and rectal temperature, blood pressure and heart rate were all significantly increased.

It is well known that prostaglandins (PGs) of the E series applied into the cerebroventricle inhibited water diuresis[1] and increased plasma and urinary concentrations of antidiuretic hormone (ADH)[2,3]. The present authors demonstrated that PGE_2, when administered into the lateral ventricle in ethanol-anaesthetized rats caused diuresis followed by antidiuresis[4,5]. Centrally injected $PGF_{2\alpha}$ and PGA_2 also changed urine outflow in the rat[4,6,7]. These findings led to the concept that PGs in the central nervous system played important roles in water metabolism.

On the other hand, the endoperoxide intermediates PGH_2 and PGG_2, identified in the PG biosynthetic pathway[8–10], were found to have some biological activities in the peripheral tissues[11–13]. Hitherto, there have been few reports concerning the effects of PGH_2 or its analogues on the central nervous system. In this study, therefore, we have investigated the effects of intracerebroventricularly (i.c.v.) administered PGH_2 on urine outflow in ethanol-anaesthetized rats. Since it has already been reported that central PGE[14–17]and PGH_2 analogues[18] changed body temperature,