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Study with Positron Emission Tomography of the Osmotic Opening of the Dog Blood-Brain Barrier for Quinidine and Morphine

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Abstract—A canine model was used to evaluate the possibilities offered by positron emission tomography (PET) for the study of drug distribution in the brain during altered states of the blood-brain barrier (BBB). PET was used to monitor the changes in the distribution of [^{11}C]quinidine and [^{11}C]morphine resulting from BBB-disruption by intracarotid infusion of a hyperosmolar mannitol solution. Injection of Evans blue dye allowing post-mortem evaluation of the degree of BBB-opening was used as a reference method. Brain radioactivity concentrations observed after i.v. injection of either [^{11}C]quinidine or [^{11}C]morphine were markedly increased by intracarotid mannitol infusion, whereas they were not affected by saline infusion. For both drugs a close correlation was found between the radioactivity concentrations and the degree of Evans blue staining within the brain hemispheres and within smaller regions of interest corresponding to quadrants of a hemisphere. This parallelism between the findings for radioactivity concentrations and Evans blue staining suggests that PET allows the detection of in-vivo changes in brain distribution of drugs resulting from alterations of the BBB permeability.

The transfer of drugs from blood plasma into the brain is restricted by the blood-brain barrier (BBB). In-vivo study of the factors affecting the passage of drugs through this barrier is technically difficult. Positron emission tomography (PET) allows a non-invasive, in-vivo monitoring of the distribution of labelled compounds. We used this technique to evaluate the effects of osmotic opening of the BBB with mannitol (Rapoport et al 1980) on the distribution in dog brain of [^{11}C]quinidine and [^{11}C]morphine, drugs with limited passage through the BBB in normal circumstances (Armand et al 1969; Hartvig et al 1984; Agon et al 1988). The findings with PET for [^{11}C]quinidine and [^{11}C]morphine were compared with the extent of osmotic BBB-opening measured according to the Evans blue staining method (Rapoport et al 1972).

Materials and Methods

Animals and preparation

Adult mongrel dogs, 9·5–16 kg, premedicated with intramuscular atropine (0·1 mg kg^{-1}) and pethidine (10 mg kg^{-1}), were anaesthetized with intravenous sodium pentobarbitone (20 mg kg^{-1}). The dogs were ventilated after the administration of gallamine (2 mg kg^{-1}). Catheters were inserted in the right femoral artery for blood pressure monitoring and blood sampling, and in the left femoral vein for drug administration. For infusion of either mannitol or 0·9% NaCl (saline) solution via the internal carotid artery, the left common carotid artery was cannulated and the left external carotid artery was ligated.

Radiopharmaceuticals and positron emission tomography

[6′-*O*-Methyl^{11}C]quinidine and [*N*-methyl^{11}C]morphine were synthesized by alkylation of the corresponding desmethyl derivative with [^{11}C]methyl iodide (Van Haver et al 1985). [^{11}C]Quinidine (30–78 mCi; 1·11–2·89 GBq; n = 7) was dissolved in saline containing non-labelled quinidine, to a total dose of 1 mg kg^{-1}. [^{11}C]Morphine (16–68 mCi; 0·59–2·04 GBq) was dissolved in either a morphine solution (total dose administered 0·5 mg kg^{-1}; n = 2) or a saline solution (n = 4). A NeuroECAT positron tomograph (ORTEC) was used to study the distribution of the drug in the brain. The slice studied was perpendicular to the orbito-meatal plane and 20 mm anterior to the transaxial plane that goes through the ear bars of the head holder. The slice thickness was 16 mm and the in plane resolution equals 8·5 mm in high resolution mode (Hoffman et al 1983). Sequential scans were performed with decay compensation.

Experimental protocol

The dog was positioned with its head in the gantry of the positron tomograph. Evans blue (2% w/v; 2 mL kg^{-1}) was then injected intravenously to allow post-mortem evaluation of the integrity of the BBB. Twenty minutes later, approximately one third of the total dose of either [^{11}C]quinidine or [^{11}C]morphine was injected and four scans were performed. Nine minutes after the first drug injection, either a mannitol (25% w/v; 60 mL) or a saline (60 mL) solution was infused via the left internal carotid artery over 30 s. Two minutes later, the remaining two thirds of the dose of either [^{11}C]quinidine or [^{11}C]morphine was injected and 19 scans were taken over the next 75 min.

Data processing

Positron emission tomography. The scans performed after the first drug injection were used to ascertain the drug distribution into the brain before intracarotid infusion of either mannitol or saline; the scans obtained after the second drug injection were used to evaluate the influence of either mannitol or saline infusion.

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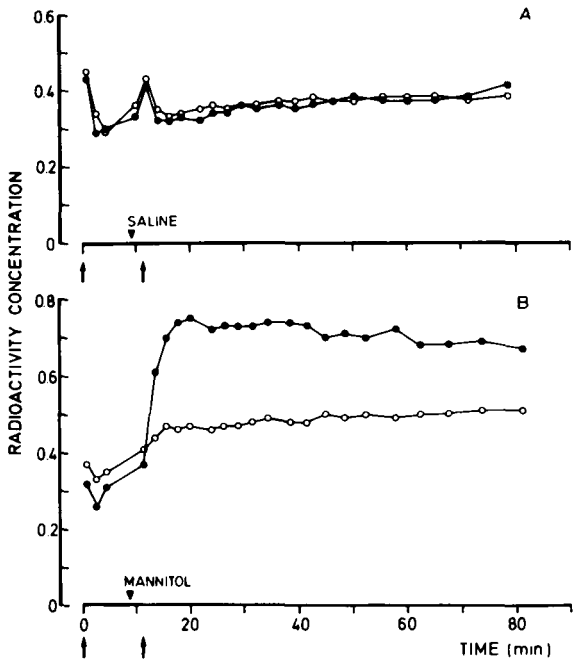

FIG. 1. Radioactivity concentration-time curves for the left (●) and right (○) brain hemispheres, after the administration of [^{11}C]quinidine. One third of the total dose was injected at time zero and the remaining two thirds 11 min later (at arrows). A saline solution (A) or mannitol solution (B) was infused between both injections (▼) via the left internal carotid artery. Relative concentrations (μCi found per mL tissue/μCi injected per g body weight) are given.

Radioactivity concentrations were calculated in two symmetrical regions of interest corresponding to the hemispheres, and also in smaller regions corresponding to the quarters of each hemisphere. Radioactivity concentrations in each region of interest are always expressed as relative concentrations i.e.

$$\frac{\mu\text{Ci found per mL tissue}}{\mu\text{Ci injected per g body weight}}$$

(Woodard et al 1975).

An area under the radioactivity concentration-time curve (AUC) was calculated from the second drug injection on to the end of the experiment.

Visual inspection of Evans blue staining. Post-mortem, the anatomical slice corresponding to the PET-slice was dissected and the degree of staining in the regions of interest was evaluated by seven observers who were not aware of the treatment applied. Scoring was done as follows: grade 0, no staining; Grade 1+, just noticeable staining; Grade 2+, moderate blue staining; and Grade 3+, dark blue staining. Results for each hemisphere are presented as the sum of the scores by the seven observers.

Results

[^{11}C]Quinidine

Radioactivity concentration-time curves for the large regions of interest, corresponding to the brain hemispheres, obtained in representative experiments with intracarotid

Table 1. Radioactivity concentrations (expressed as AUC) and Evans blue staining (expressed as sum of the observers' score), for dogs given [^{11}C]quinidine after intracarotid infusion of either mannitol (n = 3) or saline (n = 4). Values are given for the ipsilateral (ipsi) and for the contralateral (contra) hemisphere.

	Mannitol					Saline			
	Ipsi		Contra			Ipsi		Contra	
Large ROIs*	AUC	Ev.Blue	AUC	Ev.Blue		AUC	Ev.Blue	AUC	Ev.Blue
# 114	46·40	17	31·97	2	# 115	24·26	2	25·04	1
# 117	58·40	20	47·06	10	# 116	23·27	0	23·65	0
# 124	35·18	15	27·81	11	# 125	26·89	2	27·00	0
					# 126	21·92	0	20·91	0
Small ROIs**									
# 114 a	54·11	18	43·46	8	# 115 a	28·03	1	26·25	2
b	40·46	16	33·18	0	b	23·63	2	26·18	0
c	54·35	19	33·94	0	c	25·10	2	26·26	2
d	40·69	15	26·90	0	d	24·04	1	27·53	1
# 117 a	58·38	20	57·71	16	# 116 a	25·65	0	25·15	0
b	52·78	20	48·96	1	b	25·46	0	26·42	0
c	65·96	20	48·11	15	c	25·58	0	26·30	0
d	62·95	20	42·91	10	d	23·38	0	25·02	0
# 124 a	42·37	14	31·01	20	# 125 a	29·73	0	26·80	0
b	27·56	20	30·36	1	b	20·64	0	29·82	0
c	43·42	14	30·45	13	c	30·34	0	25·96	0
d	34·75	13	26·79	11	d	20·60	7	27·73	0
					# 126 a	24·65	0	21·54	0
					b	21·54	0	22·60	0
					c	22·64	0	18·41	0
					d	19·40	0	22·88	0

*large ROIs corresponding to the hemispheres.
**small ROIs corresponding to quarters of each hemisphere, where a, b, c, d is, respectively, the left and right upper and the left and right lower quadrant.

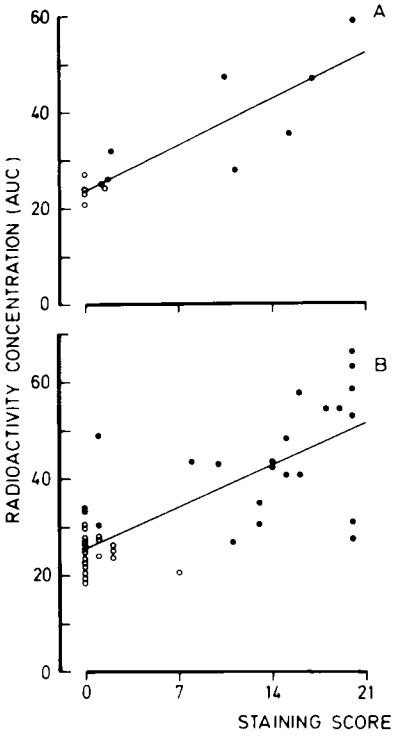

FIG. 2. Correlation between the scores for Evans blue staining and the radioactivity concentrations for [^{11}C]quinidine calculated as AUC for brain hemispheres (A) and for quadrants of brain hemispheres (B), in dogs infused with either saline (○) or mannitol (●). The coefficients of correlation are 0·873 ($P < 0·001$) and 0·779 ($P < 0·001$) for the large and small regions of interests respectively.

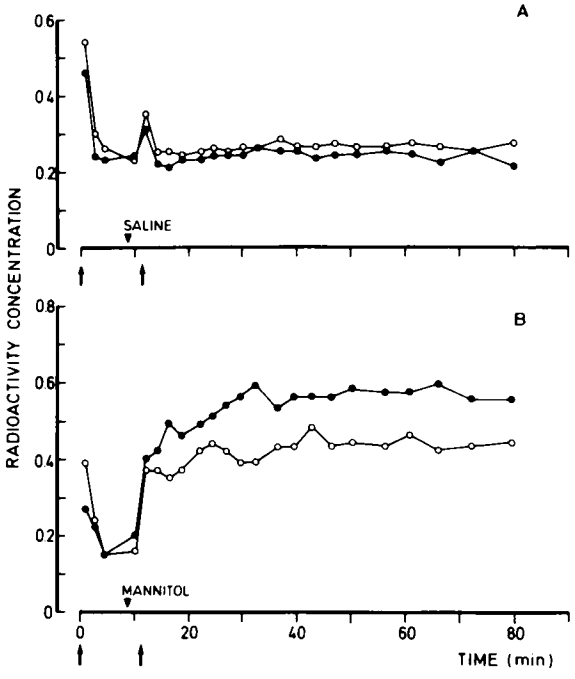

FIG. 3. Radioactivity concentration-time curves for the left (●) and right (○) brain hemispheres, after the administration of [^{11}C]morphine. One third of the total dose was injected at time zero and the remaining two thirds 11 min later (at arrows). A saline solution (A) or mannitol solution (B) was infused between both injections (▼) via the left internal carotid artery. Relative concentrations (μCi found per mL tissue/μCi injected per g body weight) are given.

infusion of mannitol and saline, are illustrated in Fig. 1. The results in all dogs, for both large and small regions of interest, are summarized in Table 1.

The radioactivity concentrations found in both brain hemispheres after the first dose of [^{11}C]quinidine, were similar in all seven experiments. The radioactivity concentrations, i.e. relative concentrations, after the second drug injection remained essentially unchanged in the animals in which saline was infused via the left internal carotid artery: no asymmetry between the two brain hemispheres was

Table 2. Radioactivity concentrations (expressed as AUC) and Evans blue staining (expressed as sum of the observers' score), for dogs given [^{11}C]morphine after intracarotid infusion of either mannitol (n = 3) or saline (n = 3). Values are given for the ipsilateral (ipsi) and for the contralateral (contra) hemisphere.

	Mannitol					Saline			
	Ipsi		Contra			Ipsi		Contra	
Large ROIs*	AUC	Ev.Blue	AUC	Ev.Blue		AUC	Ev.Blue	AUC	Ev.Blue
# 129	35·76	16	27·66	8	# 130	14·14	0	13·34	0
# 135	31·67	11	29·35	8	# 134	13·14	0	13·78	0
# 138	27·32	5	36·23	12	# 136	15·60	1	17·04	1
Small ROIs**									
# 129 a	48·45	16	36·09	19	# 130 a	18·93	0	19·37	0
b	43·20	17	27·88	0	b	12·95	0	20·39	0
c	40·15	17	28·78	5	c	16·02	0	14·73	0
d	28·47	13	26·42	9	d	13·27	0	12·34	0
# 135 a	35·95	13	47·63	20	# 134 a	19·75	0	14·52	0
b	41·33	17	22·16	1	b	12·82	0	13·39	0
c	27·09	5	39·76	9	c	14·79	0	18·83	0
d	38·20	8	20·26	1	d	10·31	2	13·22	2
# 138 a	37·67	2	40·93	20	# 136 a	16·93	1	21·54	2
b	25·94	7	36·58	1	b	13·49	1	15·92	0
c	34·58	4	38·01	20	c	19·59	1	20·74	1
d	26·26	5	25·99	9	d	17·35	1	14·19	1

* large ROIs corresponding to the hemispheres.
**small ROIs corresponding to quarters of each hemisphere, where a, b, c, d is respectively the left and right upper and the left and right lower quadrant.

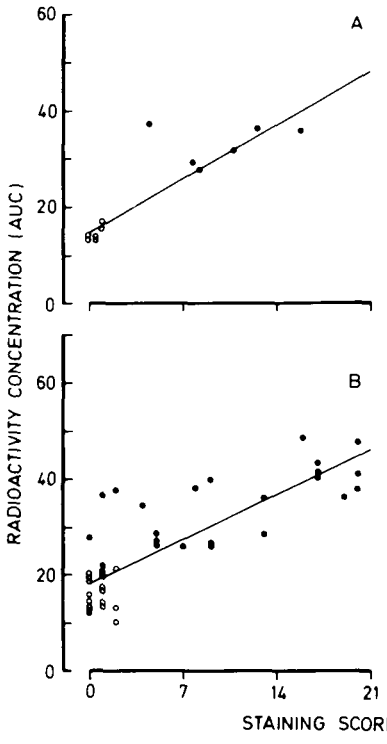

FIG. 4. Correlation between the scores for Evans blue staining and the radioactivity concentrations for [^{11}C]morphine calculated as AUC for brain hemispheres (A) and for quadrants of brain hemispheres (B), in dogs infused with either saline (O) or mannitol (●). The coefficients of correlation are 0·964 ($P < 0·001$) and 0·892 ($P < 0·001$) for the large and small regions of interest, respectively.

noticed and the small initial peak in the first scan after the second injection was similar to the small initial peak observed in the first scan after the first injection. After mannitol infusion, in the ipsilateral brain hemisphere radioactivity concentrations increased after the second drug injection to reach a new plateau, while a smaller increase was seen in the contralateral hemisphere.

No or only barely noticeable Evans blue staining of either brain hemisphere was seen post-mortem in the animals in which saline had been infused. For the animals infused with mannitol, a moderate to dark blue staining was observed in the ipsilateral hemisphere, and no or a moderate staining in the contralateral hemisphere (Table 1).

Analysis of the small regions of interest (ROI's) showed that the distribution of radioactivity concentration and the Evans blue staining within each hemisphere were not homogeneous. The coefficients of correlation (linear regression) between AUC and Evans blue staining were 0·873 ($P < 0·001$) and 0·779 ($P < 0·001$) for the large and small ROI's, respectively (Fig. 2).

[^{11}C]Morphine

Radioactivity concentration-time curves for the large brain hemispheres obtained in representative experiments are illustrated in Fig. 3. The results are summarized in Table 2.

Here too, brain radioactivity concentrations after the first dose were similar in all experiments. Similar patterns as in the experiments with [^{11}C]quinidine were observed: after intracarotid saline infusion, radioactivity concentrations remained unchanged and no asymmetry was noticed; after mannitol infusion, the radioactivity concentrations in the ipsilateral brain hemisphere increased. However, the brain radioactivity concentrations before and after mannitol were both smaller than for quinidine.

Analysis of the Evans blue staining did not show any staining in the animals infused with saline; in the animals infused with mannitol there was a moderate to dark blue staining in both hemispheres. In one of the three dogs (#138; Table 2), staining was more pronounced in the contralateral than in the ipsilateral hemisphere.

Analysis of the small ROI's showed, as for quinidine, heterogeneity within each hemisphere for radioactivity distribution as well as for Evans blue staining.

The coefficients of correlation between AUC and Evans blue staining were 0·964 ($P < 0·001$) and 0·892 ($P < 0·001$) for the large or small ROI's, respectively (Fig. 4).

Discussion

The aim of the experiments presented was to investigate whether PET can contribute to the study of changes in the passage of drugs from blood plasma to the brain. This passage is restricted by the BBB and depends on the physicochemical properties of the molecules (Oldendorf 1974).

For both drugs, brain radioactivity after the first injection, before the intracarotid infusion, was low, as already described for quinidine by Agon et al (1988) and for morphine by Oldendorf et al (1972) and Hartvig et al (1984). Quinidine is approximately 90% ionized at physiological pH and is highly protein bound in the dog (Ochs et al 1980); morphine is only bound to a limited extent (Baggot & Davis 1973) but is poorly lipid-soluble (Jaffe & Martin 1985).

The small initial radioactivity peak observed immediately after injection of quinidine and morphine is probably due to the very high blood radioactivity concentrations found during the early distribution phase (unpublished results).

When the second drug injection was given after intracarotid saline infusion, the distribution of radioactivity was similar to that after the first injection, indicating that the transient increase of intravascular pressure by itself had no sustained effect on the passage of the drug through the BBB. After intracarotid infusion of the hyperosmolar mannitol solution, the passage of both quinidine and morphine was, however, markedly increased. Hyperosmolar solutions open vessels by shrinkage of the endothelial cells and so disrupting the tight junctions between these cells (Rapoport 1976). The brain concentrations of quinidine and morphine achieved after mannitol were still lower than those we observed previously using the same PET-technique for [^{11}C]antipyrine and [^{11}C]imipramine without manipulation of the BBB (Agon et al 1988); this can probably be explained by the fact that the total area constituted by the mannitol-opened tight junctions is small, compared with the area available for the passage of lipid-soluble drugs, which can cross cell membranes.

Injection of Evans blue dye allowed post-mortem evaluation of the staining of the brain and so of the degree of barrier opening for the albumin-Evans blue complex (Freedman & Johnson 1969; Rapoport et al 1972). Staining of the brain was observed in all animals after mannitol. For both

quinidine- and morphine-treated dogs, a good correlation between radioactivity concentrations and Evans blue staining was found. Rapoport et al (1980) proposed that osmotic barrier opening at a given vascular site might be an all-or-none phenomenon, affecting equally the passage of small and larger molecules. Our findings for the Evans blue-albumin complex and for two drugs with different physicochemical properties and different plasma protein binding seem in accordance with this view.

In all experiments where mannitol was infused, a clear asymmetry was seen between the hemispheres. While in most of the experiments the highest increase was seen in the ipsilateral hemisphere, one animal showed a higher increase at the contralateral site. This is in keeping with the known variability in the anatomy of the brain blood supply in the dog (Neuwelt et al 1980).

Within each hemisphere the distribution of both radioactivity and Evans blue staining was not homogeneous. This is not only due to the differences in mannitol perfusion in different brain regions, but also to other factors such as regional differences in the structure of brain tissues. Analysis of the small ROI's, corresponding to the quadrants of each hemisphere, confirmed this heterogeneity; but here too a good correlation between radioactivity concentrations and Evans blue staining was found. The poor resolution attainable with PET and the small size of the dog brain did not allow a more detailed study of regional drug distribution.

It should be emphasized that PET provides only information about radioactivity distribution. The possible in-vivo metabolism of the labelled compound constitutes a limitation for this technique. This is illustrated by preliminary results of rapid analysis, as described by Garrett & Gürkan (1974), for morphine in plasma. In these experiments, 15 min after the administration of [^{11}C]morphine, less than 25% of radioactivity corresponded to unchanged [^{11}C]morphine and the remainder to more hydrophilic compounds (Agon et al unpublished results).

The parallelism between the findings for radioactivity concentrations and Evans blue staining in the present study indicates that PET allows the detection 1 cm in-vivo of changes in the brain distribution of drugs which result from alterations of the BBB permeability. Kessler et al (1984) reported the in-vivo study of BBB-permeability with PET and a radiotracer, [^{68}Ga]EDTA, selected for its normally very low permeability through the BBB. The present study extends this report by illustrating that the radiotracer selected can not only be a tool but also in itself be the subject of pharmacological studies in altered states of the BBB.

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