Passage through the blood-brain barrier of thyrotropin-releasing hormone encapsulated in liposomes

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The elucidation of the mechanism which underlies the blood-brain barrier would represent major progress, chemical substances could then be introduced into the brain without interfering with the natural physiological and anatomical barriers. Since their introduction in 1965 (Davson 1976) liposomes (phospholipid bilayer vesicles) have been used in the study of membrane transport processes and more recently as a transport vehicle to introduce liposome-encapsulated molecules and drugs into cells (Bangham et al 1965; Gregoriadis & Ryman 1972; Magee & Miller 1972; Segal et al 1976).

The results obtained during the course of various studies involving the crossing of the blood-brain barrier by liposomes under normal physiological conditions seem contradictory (Rahman et al 1974; Ryman et al 1978). Rahman et al (1974) reported that after the injection of ^{14}C-EDTA encapsulated in neutral liposomes, 0·62% of the initial dose was in the brain after 5 min, whereas from a similar dose of 'free' ^{14}C-EDTA only 0·16% remained, the blood concentrations for liposomes and free EDTA being respectively 27·6 and 13·2%. They did not prove, however, that the EDTA had actually entered the brain. The results could have been attributed to adsorption to the endothelial cells, lining the brain capillaries.

Again, Metcalf (1974) showed that the intraventricular injection of thyrotropin-releasing hormone (TRH, p.glu.his.pro.NH_2) in cats causes a dose-dependent drop in body temperature, whereas its intravenous administration did not have such an effect. In rabbits, however, an intraventricular injection of TRH caused a rise in body temperature.

We have investigated the influence of liposomes on passage of the blood brain barrier by TRH by measuring the uptake of ^{131}I-TRH in mice and the 'pyrogenic' effect of TRH in rabbits.

In mice ^{131}I-TRH and ^{131}I-TRH liposomes respectively were injected intravenously. After 3 h the brain was removed and its radioactivity measured.

To eliminate intravascular blood radioactivity, similar experiments were performed after body perfusion with phosphate buffered saline, containing 0·1% procaine. In addition, the blood volume of the brain was measured by means of [^{131}I]albumin, which does not cross the blood-brain barrier.

A mean brain blood volume of 32 μl (n = 60) was estimated, being roughly 3·5% of the wet weight of the brain.

The difference between perfused and non-perfused mice was not significant. Three h after the injection of [^{131}I]albumin enclosed in neutral liposomes the brain activity in a group of perfused mice was only slightly less than that in a non-perfused group: 0·40% ± 0·194 versus 0·44% ± 0·110.

In all these preliminary experiments with [^{131}I]-albumin the brain uptake in mice treated with neutral liposomes loaded with [^{131}I]albumin was about twice that in mice injected with radioactive albumin only, Hence we conclude that there is a small but still net brain-uptake of albumin which should be attributed to a liposome effect. Similar results were obtained with TRH-liposomes (Table 1). Here, after 3 h the brain uptake of liposome-transported TRH was between 0·28% and 0·44% of the dose injected, a definitely higher amount than when 'free' TRH was injected (see Table 1). Moreover, neutral liposomes appear to pass the blood-brain barrier better than positive or negative liposomes.

In our mice the brain uptake of the neutral TRH-liposomes is 0·44% (minus the control value of free TRH: 0·12% the uptake is 0·32%). The effect of TRH encapsulated in liposomes on the rise in body temperature (Δt) in rabbits was investigated. A rise would indicate that sufficient amounts of TRH had passed the blood-brain barrier to stimulate 'temperature' centres probably located in the hypothalamus and mesencephalon (Goldmann 1913).

The liposome lipids were checked for pyrogenicity using the 'Limulus assay'. Positive liposomes were not used because they can cause epileptic seizures and cerebral tissue necrosis due to the toxicity of stearylamine.

'Free' TRH and TRH encapsulated in neutral and negative liposomes were injected intravenously into animals routinely used for pyrogenicity testing under identical standard conditions. Free TRH and control ('empty') liposomes did not cause a significant change in body temperature (Table 2). 20–75 min after injection of neutral or negative TRH-loaded liposomes, a marked

Table 1. The uptake of radioactive TRH encapsulated in negative positive and neutral liposomes compared with the brain uptake of 'free' ^{131}I-THR in mice.

i.v. injection of	in the brain after 3 h (% of dose (s.d.) of mean)			P*
free TRH	0·12%	(0·059)	n = 20	< 0·001
negative (TRH) liposomes	0·28%	(0·083)	n = 5	< 0·05
positive (TRH) liposomes	0·39%	(0·128)	n = 10	< 0·01
neutral (TRH) liposomes	0·44%	(0·104)	n = 10	

* According to the Student's *t*-test, compared with neutral liposomes. The lipid composition of the liposomes is given in Table 2. Free TRH uptake differs significantly from pos. and neg. liposomes with a *P*-value ⩽ 0·01.

* Correspondence.

rise in body temperature was observed (Table 2) with a Δt of 1·5 °C and 0·3 °C respectively. The rise was much more pronounced after the injection of neutral liposomes compared with negative liposomes.

These results might be explained as being the result of a facilitation of the passage of the blood-brain barrier by less polar particles. However, other experiments, such as our mice experiments, do not support this explanation. Jonah et al (1975) reported that the uptake of positive liposomes from the brains and lungs was 2–4 times higher than that of negative liposomes This difference in results might be due to a difference in the size of the liposomes used.

The rise in body temperature in the rabbit cannot be attributed to a TSH effect on the thyroid gland because the temperature changes after intravenous injection of a high dose of 'free' triiodothyronine (T3), namely 125 μg, was within the normal range.

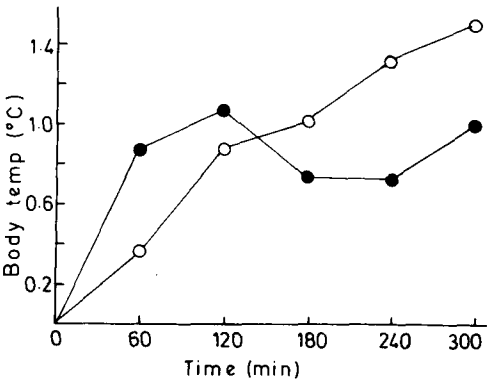

FIG. 1. Rise of body temperature after i.v. inoculation of rabbits with TRH encapsulated in liposomes (see also Table 2). (●) 125 μg TRH encapsulated in negative liposomes. (n = 3). (○) 45 μg TRH encapsulated in neutral liposomes. (n = 3).

Table 2. Maximum temperature rise of rabbits during 5 h after inoculation.

Inoculation (i.v.)		ΔT(° C)
Exp. 1— Negative liposomes)*		
encapsulated	0 μg TRH	0·3 (n = 3)
,,	10 μg TRH	0·4 (n = 3)
,,	40 μg TRH	0·3 (n = 3)
,,	125 μg TRH	1·2 (n = 3)†
'empty' liposomes with 125 μg free TRH		0·4 (n = 3)
'empty' liposomes and 125 μg TRH i.v. 5 min later		0·6 (n = 3)
Exp. 2— Neutral liposomes)**		
encapsulated	0 μg TRH	0·5 (n = 3)
,,	10 μg TRH	0·6 (n = 3)
,,	45 μg TRH	1·5 (n = 3)†
,,	125 μg TRH	1·2 (n = 3)†
'empty' liposomes with 125 μg free TRH		0·5 (n = 6)
Exp. 3—	10 μg TRH	0·2 (n = 6)
	50 μg TRH	0·3 (n = 6)
	100 μg TRH	0·3 (n = 6)
	125 μg TRH	0·1 (n = 3)
	100 μg trijodothyronine (T3)	0·2 (n = 5)

* formula: P.C.: Chol: PS = 53: 19: 10 (Phosphatidylcholine, Cholesterol, Phosphatidylserine) (10–12% TRH encapsulated). To prepare liposomes with a high percentage encapsulation one has to start with 60 mg total lipid and 2 ml saline with or without TRH. After 2 h sonicating of this solution in a test tube (Bransonic B 220–1 phase 120 W–50 Ke, cleaning metal waterbath) at 21 °C, the liposomes were dialysed against saline and were ready for use after 24–30 h.

‡ average liposomal size smaller than 1·5 μm: measured by microscope.

** formula: PC: Chol = 3:1 (2–5% TRH encapsulated).

† ΔT is significantly higher than after an injection with 'empty' liposomes of the same composition or the same amount of 'free' TRH.

If we assume that the uptake of liposomes after 3 h is about the same in mice and rabbits then roughly 0·14 μg TRH (0·3% of e.g. 45 μg) might have been diffusely spread throughout the brain. This is a total brain dose almost equal to the intraventricular TRH dose of 0·5–1·0 μg used in the rabbit experiment in the study of Metcalf (1974).

From our experiments it may be concluded that liposomes containing TRH can enter the brain. Similar studies could be performed with liposomes containing different neuropeptides.

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REFERENCES

Bangham, A. D., Standish, M. M., Watkins, J. C. (1965) J. Mol. Biol. 13: 238–252

Davson, H. (1976) J. Physiol. 255: 1–28

Dunnick, K. J., Kallman, R. F., Kriss, J. P. (1976) Biochem. Biophys. Res. Commun. 73: 619–624

Goldmann, E. E. (1913) Abh. preuss. Akad. Wiss. Phys. Math. Kl. 1: 1–60

Gregoriadis, G., Ryman, B. E. (1972) Biochem. J. 129: 123–133

Jonah, M. M., Cerny, E. A., Rahman, Y. E. (1975) Biochim. Biophys. Acta 401: 336–348

Magee, W. E., Miller, O. V. (1972) Nature (London) 235: 339–341

Metcalf, G. (1974) Ibid. 252: 310–311

Rahman, Y. E., Rosenthal, M. W., Cerny, E. A., Moretti, E. S. (1974) J. Lab. Clin. Med. 83: 640–647

Ryman, B. E., Jnekes, R. F., Jeyasingh, K., Osborne, M. P., Patel, H. M., Richardson, V. J., Tattersall, M. H. N., Tyrell, D. A. (1978) Ann. New York Acad. Sci. 308: 281–307

Segal, A. W., Gregoriadis, G., Lavender, J. P., Tarin, D., Peter, T. J. (1976) Clin. Sci. Mol. Med. 51: 421–425

Central hypotensive effect of γ-aminobutyric acid in anaesthetized dogs

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γ-Aminobutyric acid (GABA) is widely distributed in the central nervous system (Berl & Waelsh 1958) and is known to produce hypotension when injected intravenously (Elliot & Hobbiger 1959; Stanton 1963) or intracisternally in rabbits (Takahashi et al 1959). Dhumal et al (1976) have reported that GABA regulates body temperature through release of putative transmitters. It is likely that GABA may have a role in centrally mediated regulation of blood pressure.

In the present study the effects of intraventricularly administered GABA were examined on the blood pressure of anaesthetized dogs. An attempt has also been made to elucidate the mechanism by which GABA affects the blood pressure. Mongrel dogs (6–10 kg, either sex) were anaesthetized with intravenous pentobarbitone sodium (30 mg kg^{-1}) and carotid blood pressure recorded. The details of perfusion of the cerebral ventricles from ventricular to aqueductal cannula and the intraventricular injections of drugs have been reported elsewhere (Dhumal et al 1974).

Optimal hypotensive effects were obtained with a 1 mg dose of GABA injected in volume of 0·5 ml. The reduction in blood pressure (mean ± s.e.) was 30 mm ± 3 mm Hg (n = 7) and the effect lasted for 10–15 min. Repeated administration of the same dose produced a smaller decrease in blood pressure than first. Intraventricular injection of 0·5 ml of artificial CSF had no effect on blood pressure.

In three experiments, noradrenaline (NA) 500 ng or adrenaline (A) 1 μg, administered intraventricularly also produced hypotension. Perfusion of the ventricles with phentolamine (20 μg ml^{-1}) for 30 min did not produce any effect on blood pressure, but it reduced the hypotensive effects of A and NA completely, and of GABA by about 50% (Fig. 1b). In the present experiments, administration of cocaine 1 mg, intraventricularly before injection of GABA, resulted in a loss of the hypotensive response to GABA (Fig. 1c).

The NA content in cerebrospinal fluid (c.s.f.) was estimated as described by Dhumal et al (1974). In these experiments, cocaine (1 mg) intraventricularly, increased the amount of NA in the cerebral effluent. When NA (500 ng) was injected, after cocaine, there was a significant ($P < 0.001$) increase in content of NA in the c.s.f. compared with the NA content in the effluent after i.c.v. NA (500 ng) injection alone; this indicated a block of the uptake of exogenously injected and endogenously released NA by cocaine.

The release of NA after GABA was, however, significantly ($P < 0.001$) less after cocaine (Fig. 2). Pretreatment with reserpine (0·3 mg kg^{-1} i.m., 72 h before) also abolished the hypotensive response of GABA and reversed it to a hypertensive response which was not studied further. In four experiments, where ventricles were perfused with calcium-free artificial c.s.f. containing sodium edetate (0·37 mg ml^{-1})

* Correspondence.

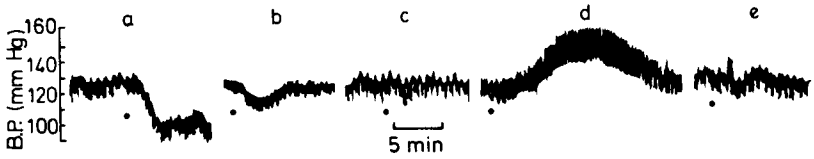

FIG. 1. Arterial blood pressure response obtained from separate dogs anaesthetized with pentobarbitone sodium (30 mg kg^{-1} i.v.). Responses to GABA (1 mg) i.c.v. (○) were studied after various pretreatments (a) control hypotensive response of GABA; (b) after perfusion with artificial cerebrospinal fluid containing phentolamine (20 μg ml^{-1}) at a rate of 0·4–0·5 ml min^{-1} from the ventricular to the aqueductal cannulae for 30 min. Hypotensive response is inhibited; (c) after pretreatment with cocaine (1 mg) i.c.v., hypotensive response is inhibited; (d) in reserpinized dog, hypotensive response is converted into a hypertensive response; (e) ventricles perfused with calcium-free solution resulted in a loss of hypotensive response.