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# Hormonal influence on the permeability of the blood–brain barrier: effect of an analog of adrenocorticotropic hormone, β 1–24 corticotrophin

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Regional unidirectional transport of α-aminoisobutyric acid (AIB) (mol. wt.: 104) and sucrose (mol wt.: 342) which have a low permeability across the intact endothelium was investigated in brain of rats either treated with synacthène: an analog of ACTH, tetracosactide retard (β-1–24 corticotrophin) or in brain of placebo-treated controls. Three days treatment with synacthène, reduced the rate of influx of AIB and sucrose in most of the brain regions studied especially in thalamus, hypothalamus, cortex, and caudate nucleus without affecting the vascular compartment. The brainstem, cerebellum and white matter were less affected. These experimental findings may suggest that ACTH exhibits significant influence on hormonal regulation of blood–brain barrier permeability. Thereby such a regulation may involve the entry of polar compounds into the CNS and may influence the central effects of diffusion-limited drugs.

Steroids have been used extensively in treating patients with brain tumours and were shown to have beneficial effects in reducing cerebral edema [7, 22]. Initial laboratory studies demonstrated that steroids decrease edema observed in experimentally-induced hypertension [10, 23], tumour [16], seizure activity [3], cerebral infarction [5] and osmotic blood–brain barrier (BBB) disruption [12]. It has also been shown that adrenal corticoids modify the permeability surface-area product (PA) for water in the cerebral cortex of rats [17, 18] and the normal permeability of cerebral vessels to horseradish peroxidase in mice [9]. More recently, the studies in our laboratory clearly demonstrated that the administration of a glucocorticoid, dexamethasone, produced a decreased cerebrovascular permeability of α-aminoisobutyric acid (AIB) and sucrose while withdrawal of the drug or administration of ethinylestradiol resulted in increased permeability which suggests that adrenal corticoid hormones may play an important role in regulating the BBB permeability [24, 26]. Furthermore, Long and Holaday [11] have reported that bilateral adrenalectomy but not medullectomy increased the penetration of $^{125}$I-labelled bovine serum albumin (BSA) into brain, and that this effect was reversed by administration of corticosteroids.

These observations and findings by Rudman and Kutner [19] that suppression of ACTH secretion or intracisternal administration of ACTH (both are associated with a decreased secretion of corticosteroids) increased the penetration of albumin, sucrose or mannitol from blood into CSF and brain led us to speculate that pituitary-adrenal axis might also play an important role in the regulation of permeability of undisrupted normal brain microvasculature. Therefore in this present report we sought to obtain indirectly some information concerning an interaction along the pituitary adrenal axis in the regulation of permeability of brain microvasculature by treating rats with an analog of ACTH, synacthène: tetracosactide retard (β-1–24 corticotrophin) (Laboratoires Ciba-Ceigy, France).

The method and experimental protocol for the measurement of cerebrovascular permeability are the same as those used previously except that synacthène replaced dexamethasone [24]. The study was carried out on two groups of animals, treated either with saline (placebo) or drug (i.p. 16 μg/kg, 4 μg/rat) for 3 days before the permeability measurements.

The regional cerebrovascular permeability was determined simultaneously for sucrose and AIB with the [$^3$H] or [$^{14}$C] form of compounds. [$^{14}$C]sucrose (340 mCi/

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mmol) was obtained from Amersham International (UK) and [$^3$H]AIB (33.5 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). 0.3 ml of isotonic saline containing 25 $\mu$Ci [$^3$H]AIB and 5 $\mu$Ci [$^{14}$C]sucrose was injected intravenously in synacthène-treated rats or in control rats. 10 min after the injection of the radiotracers the brain was quickly removed from the skull and dissected into specific regions. Whole blood, arterial plasma and weighed regions of the brain were prepared for counting the radioactivity as previously described [24]. Regional unidirectional blood-to-brain transport constants ($K_i$) were calculated for [$^3$H]AIB and [$^{14}$C]sucrose from the tissue and plasma radioactivity data using the following equation originally developed by Ohno et al. [13].

$$K_i = \frac{C_{br}(T) - V \cdot C_{wb}(T)}{\int_0^T C_{pl}\, dt} \quad (1)$$

where $C_{br}$ is the amount of tracer in brain per unit mass of tissue, (dpm/g$^{-1}$) at the time $T$, $T$ is the duration of the experiment (min), $C_{pl}$ is the arterial plasma concentration of the tracer (dpm/ml$^{-1}$), $V$ is the regional blood volume/g$^{-1}$ and $C_{wb}$ is the tracer concentration in final whole blood (dpm/ml$^{-1}$).

For calculation and to explore the possibility that synacthène may have induced changes in the vascular compartment, the amount of tracer within the vascular space (blood volume, $V$) was measured in control and synacthène-treated rats. Regional blood volume was determined from sucrose space as the value defined as: (dpm/g$^{-1}$) brain/(dpm/ml$^{-1}$) whole blood × 100 at the time of death 2 min after the i.v. injection of [$^{14}$C]sucrose.

Statistical analysis was performed using Student's $t$-test. One-way analysis of variance was used to compare the groups followed by Student's $t$-test when overall difference was significant. All values are means ± S.E.M., unless the overall difference was significant. All values are means ± S.E.M., unless otherwise noted significance was taken as $P < 0.05$.

Table I presents the mean regional [$^{14}$C]sucrose space in control and synacthène-treated rat brains that were used to calculate $K_i$ by Eqn. 1. Cerebrovascular space values measured for sucrose were found to be similar in both animal groups treated by either placebo or drug. In control rat brain the sucrose space was ranged from 1.05% (striatum) to 3.03% (bulbus olfactorius). Regional sucrose space did not change significantly ($P > 0.05$) in any of the brain regions studied after treatment with synacthène. It was between 1.08% (striatum) and 2.86% (bulbus olfactorius).

The unidirectional blood-to-brain transport constants ($K_i$) for AIB and sucrose calculated from Eqn. 1 in rats treated with drug or saline are shown in Table II. The 10 min average mean of $K_i$ for AIB in control rats was about 2.11 ml/g$^{-1}$/min$^{-1}$ × 10$^3$, whereas the $K_i$ for sucrose was 0.64 ml/g$^{-1}$min$^{-1}$ × 10$^3$.

Moreover, the $K_i$ for sucrose showed a high degree of correlation with the regional permeability pattern of AIB and when compared to that of AIB was lower in every region of the brain of all animals as an expected consequence of the limited diffusibility of sucrose.

A significant decrease in $K_i$ by 35% and 25% for both [$^3$H]AIB and [$^{14}$C]sucrose, respectively, was observed in almost all brain regions studied in rats pretreated for 3 days with synacthène. The effect of synacthène was variable depending on the region and was most clear in thalamus, hypothalamus, cortex and caudate nucleus. Permeability appeared to be less affected in the brainstem, cerebellum and white matter after treatment with synacthène.

The cerebrovascular endothelial cells contain tight junctions and have been shown to block the transport of water soluble substances that have no carrier (such as sucrose). In the absence of facilitated transport mechanisms, the rate of blood–brain exchange of sucrose as well as most other substances depends mainly on the molecules affinities for water and membrane lipid as well as the nature of cellular surfaces which must be traversed

TABLE I

REGIONAL [$^{14}$C]SUCROSE SPACES

Values are means ± S.E.M.. Data were calculated as (dpm/g$^{-1}$ brain)/(dpm/ml$^{-1}$ whole blood) at 2 min after intravenous injection of [$^{14}$C]sucrose

| Brain region | Space (%) | |
|---|---|---|
| | Control ($n$ = 4) | Synacthene-treated ($n$ = 6) |
| Olfactory bulb | 3.03 ± 0.14 | 2.86 ± 0.12 |
| Caudate nucleus | 1.05 ± 0.08 | 1.08 ± 0.07 |
| Hippocampus | 1.15 ± 0.08 | 1.12 ± 0.10 |
| Frontal lobe | 1.95 ± 0.07 | 1.90 ± 0.12 |
| Occipital lobe | 2.08 ± 0.10 | 2.12 ± 0.15 |
| Thalamus and hypothalamus | 1.70 ± 0.07 | 1.74 ± 0.14 |
| Superior colliculus | 2.29 ± 0.12 | 2.19 ± 0.14 |
| Inferior colliculus | 2.06 ± 0.13 | 2.29 ± 0.19 |
| Cerebellum | 1.99 ± 0.11 | 1.83 ± 0.06 |
| Pons | 2.09 ± 0.15 | 2.16 ± 0.09 |
| Medulla | 1.80 ± 0.10 | 1.95 ± 0.09 |
| Midbrain | 1.95 ± 0.20 | 2.00 ± 0.12 |
| Parietal lobe | 1.68 ± 0.09 | 1.74 ± 0.07 |
| White matter (corpus callosum) | 1.20 ± 0.13 | 1.19 ± 0.10 |

[15]. It is a common approach in the quantitative assessment of tracer penetration into brain to inject a poorly permeating tracer intravenously and to relate parenchymal radiotracer uptake to time-integrated plasma level. This approach requires an intravascular correction that the amount of solute within the vascular space must be measured. An intravascular correction is relatively simple, but requires the use of a vascular reference substance. For solutes with low permeabilities that distribute predominantly in the plasma over a short period (a condition well satisfied for sucrose and AIB) [1, 4] the selection of an intravascular space marker is usually not a problem. Ideally, the vascular volume tracer should be equal in size to that of the test tracers or the substance itself [1, 20]. An intravascular correction was applied to present data obtained from 2-min experiments in the rat with sucrose.

Since the blood volume was relatively unaffected by treatement with synacthène, and the CBF was not affected by treatment with an ACTH/MSH$^{4-9}$ analog [8] it is possible to conclude that as the capillary surface-area remained relatively constant the observed changes in the blood-to-brain transport reflect the alteration of the blood–brain barrier permeability processes. In other words the obtained low $K_i$ in synacthène-treated rats reflects a reduction in blood–brain barrier permeability rather than other cerebral changes affecting the amount of tracer accumulated in parenchyma. These observations are in accordance with previous studies which demonstrated that an analog of ACTH/MSH$^{4-9}$, ORG-2766 is able to reduce passive transport mechanisms in cerebrovascular endothelium without detectable changes in CBF [8]. Topographically, the relative changes in $K_i$ for sucrose and AIB varied regionally as was shown briefly by Goldman and Murphy [8]. This may have been due to true differences in regional permeability or more likely to variation in capillary surface areas associated with differing mixtures of white and grey matter [2]. Furthermore, our results also showed that there is a high degree of correlation in regional $K_i$ pattern of a non-electrolyte (sucrose) and those of a small neutral amino acid (AIB). Although both these test substances cross through the endothelial cells quite slowly and satisfy well the conditions for measuring barrier permeability when expressed in terms of unidirectional transport ($K_i$) or PA [1, 4] the ($K_i$) values for AIB in all brain regions were higher than similarly computed values for sucrose. These somewhat different uptake profiles in the transport of AIB and sucrose can be explained by a rapid uptake of AIB by brain and meningeal cells which have transport systems for

TABLE II

REGIONAL BLOOD-TO-BRAIN TRANSFER CONSTANT ($K_i$) VALUES FOR [$^3$H]AIB AND [$^{14}$C]SUCROSE IN CONTROL AND SYNACTHÈNE TREATED RATS

Values are means ± S.E.M. $n$ = 4, in control and 10 in treated rats. The $K_i$ was calculated by Eqn. 1. From the integral arterial plasma concentration and $C_{br}$ (total minus intravascular radioactivity) when the animals were killed 10 min after i.v. injection.

| | $K_i$ (ml/g$^{-1}$/min$^{-1}$ × 10$^3$) | | | |
|---|---|---|---|---|
| | [$^3$H]AIB | | [$^{14}$C]sucrose | |
| Brain region | Control | 3-Day treatment | Control | 3-Day treatment |
| Olfactory bulb | 2.99 ± 0.16 | 2.11 ± 0.18* | 1.21 ± 0.19 | 0.79 ± 0.17 |
| Caudate nucleus | 1.20 ± 0.09 | 0.90 ± 0.12* | 0.50 ± 0.01 | 0.36 ± 0.07* |
| Hippocampus | 1.58 ± 0.08 | 1.02 ± 0.13* | 0.48 ± 0.02 | 0.31 ± 0.06* |
| Frontal lobe | 2.52 ± 0.14 | 1.25 ± 0.11* | 0.72 ± 0.07 | 0.49 ± 0.07* |
| Occipital lobe | 2.04 ± 0.13 | 1.37 ± 0.12* | 0.61 ± 0.06 | 0.46 ± 0.17* |
| Thalamus | 1.63 ± 0.09 | 1.07 ± 0.10* | 0.62 ± 0.01 | 0.47 ± 0.02* |
| Hypothalamus | 3.48 ± 0.25 | 1.89 ± 0.02* | 0.59 ± 0.01 | 0.38 ± 0.13* |
| Superior colliculus | 2.20 ± 0.13 | 1.24 ± 0.16* | 0.71 ± 0.07 | 0.68 ± 0.07* |
| Inferior colliculus | 2.10 ± 0.18 | 1.56 ± 0.19 | 0.73 ± 0.06 | 0.69 ± 0.11* |
| Cerebellum | 2.08 ± 0.21 | 1.70 ± 0.17 | 0.47 ± 0.03 | 0.42 ± 0.13 |
| Pons | 2.03 ± 0.24 | 1.56 ± 0.21 | 0.63 ± 0.03 | 0.58 ± 0.08 |
| Medulla | 2.41 ± 0.20 | 1.85 ± 0.26 | 0.69 ± 0.04 | 0.59 ± 0.16 |
| Midbrain | 1.53 ± 0.13 | 1.26 ± 0.14 | 0.55 ± 0.02 | 0.51 ± 0.09 |
| Parietal lobe | 2.28 ± 0.15 | 1.42 ± 0.13* | 0.61 ± 0.06 | 0.46 ± 0.08* |
| White matter (corpus callosum) | 1.60 ± 0.10 | 1.42 ± 0.09 | 0.49 ± 0.03 | 0.47 ± 0.07 |

*Significant differences ($P < 0.05$) from control.

AIB. These cells rapidly take up and accumulate AIB once it has passed across the BBB into the interstitium of the brain. Since the meningeal cells were already eliminated during dissection their contribution to actual uptake of AIB is negligible.

Despite the vigorous investigation and consisting data on potential regulatory effect of corticosteroids, little information exists concerning the site and the mechanism of action of ACTH/MSH and some of their fragments in the processes involved in the observations mentioned above. Although the remarkable small amounts of ACTH/MSH peptides are reported to reach the brain from the systemic circulation [14, 21], one possibility is that synacthène may directly alter the transport processes located within the blood–brain barrier as it has already been shown to alter blood–cerebrospinal fluid (CSF) barrier [19]. In contrast to our report in their experiments Rudman and Kutner [19] have shown that intracisternal administration of ACTH and other melanotropic peptides increase the appearance of BSA, sucrose, inulin or mannitol in CSF while intravenously injected ACTH was relatively ineffective. However, it is clear that intracisternal administration of ACTH blocks the naturally occurring ACTH release from pituitary by a feedback effect; reduced ACTH, in turn, results in less efficient stimulation of glucocorticoid secretion from the adrenal cortex. Consequently release of glucocorticoids would be deprived as well causing an increase in the permeability of BBB. Alternatively, since the secretion of endogenous corticoids is subjected to stimulation by ACTH, in consequence of the systemic administration of synacthène, the rate of release of corticoids which reduce the permeability of BBB [17, 24, 25] would be increased. Thus, treatment with synacthène may indirectly participate in the regulation of BBB transport processes by altering the level of corticosteroids which have an important role in controlling the permeability of cerebral vessels [11, 24, 25] and furthermore suggests that pituitary-adrenal axis may regulate the permeability of the BBB.

In conclusion, such physiologic regulation may in part involve the entry of polar compounds into the CNS and the influence in the central actions of diffusion-limited drugs may be significant.


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