Drug action on adenylate kinase activity in cerebrospinal fluid of arteriosclerotic patients[1]

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Summary. A manifest presence of adenylate kinase activity in cerebrospinal fluid was found in patients with cerebral arteriosclerosis. This activity was significantly higher during treatment with co-dergocrine (Hydergin®) compared with the two periods before and after treatment.

The molecular mechanisms involved in the release of the intracellular enzyme adenylate kinase, AK, (ATP:AMP phosphotransferase, EC 2.7.4.3) into the cerebrospinal fluid (CSF) in any condition of brain cell hypoxia have been hypothetically discussed[2–5]. Briefly, the hypothesis suggests that there is a lowered adenylate charge potential due to an insufficient supply of oxygen molecules to the affected brain cells and that as a consequence the membrane electrochemical potential is lowered[2–4]. The next event is an influx of water resulting in brain cell swelling[5], and the prerequisites are present for increased leakage through the plasma membrane to the extracellular fluid. AK was found to be a sensitive marker molecule under in vivo-conditions for such cellular dysfunctions[2–5].

The present study provides further evidence in support of the hypothesis by studying the influence of co-dergocrin (Hydergin®) given per os to patients with cerebral arteriosclerosis, on the efflux of AK into the extracellular fluid as represented by CSF.

Materials and methods. 10 females (mean age 80.5 ± 2.4 years, range 77–87) were included in the study. They all had manifest clinical signs of cerebral arteriosclerosis as revealed by a progressive loss of cognitive function, fluctuant disturbances of affect and behaviour and an inability to cope effectively with normal daily living activities. They also displayed signs of local cerebrovascular lesions. Most patients showed in addition evidences of cardiosclerosis and/or arterial hypertension. The study comprised a pre-treatment period of 24 days during which samples were drawn 3 times with about weekly intervals. The next period included treatment for 2 months with 6 mg of codergocrine (Hydergin®) daily per os. During the last half of the period samples were again drawn thrice with weekly intervals. The post-treatment period of 2 months followed after the discontinuation of the treatment with Hydergin. During the last month of this period 3 (in some cases 2) more CSF samples were drawn weekly. Thus the total time of the trial comprised 20 weeks.

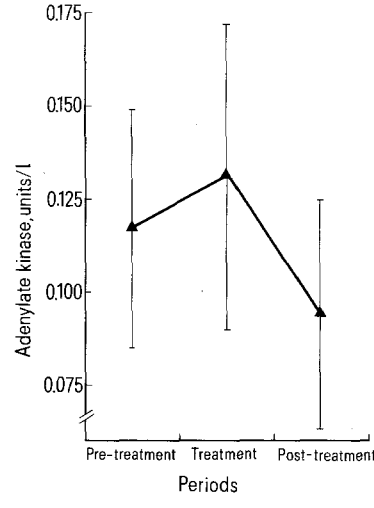
No scale for assessment of mental disability was used. The patients received their ordinary medications throughout the study and there were no changes in the nursing routine. Sampling and handling of the CSF material for analysis as well as determination of AK activity were in accordance with a previous paper[2]. Statistical analysis was carried out by the Student's two-tailed t-test with intraindividual comparison for the activities of AK obtained from the treatment as well as pre- and post-treatment period.

Results. All CSF samples, regardless of the period during which they were drawn, showed a manifest AK activity. The figure summarizes the experiments giving the mean values and SD for AK activity during the 3 different periods from all 10 patients. The figure demonstrates an increase in AK activity during the treatment period when compared with the pretreatment period. This difference is slight and not significant. However, after withdrawal of the Hydergin therapy a significant decrease of AK activity occurred ($p < 0.005$). The mean AK activity during the post-treatment period was also significantly lower than that of the pre-treatment period ($p < 0.005$) indicating a possible rebound effect on discontinuation of Hydergin therapy. A comparison was also made between the mean level of the treatment period and those of the pre- and post-treatment periods and showed a significant difference ($p < 0.05$).

The table shows that the increases and decreases of AK activities for all but 2 patients (Nos 7 and 10) were proportionally about the same and based on the initial value for each individual, resulting in similar curve profiles during the 3 periods of the trial. Linear regression analysis indicated a positive correlation between the individual values from the pre-treatment and post-treatment periods ($r = 0.82$; $p \leq 0.01$).

Individual mean AK activities in CSF of 10 patients before, during and after treatment with Hydergin. Activity is expressed in units per l of CSF. Period of pre-treatment 1–4 weeks, treatment 5–12 weeks, post-treatment 13–20 weeks

Patient number	Pre-treatment, sampling at 1–4 weeks	Treatment, sampling at 10–12 weeks	Post-treatment, sampling at 18–20 weeks
1	0.110	0.122	0.108
2	0.124	0.182	0.095
3	0.110	0.139	0.105
4	0.113	0.124	0.075
5	0.104	0.131	0.098
6	0.100	0.120	0.045
7	0.090	0.078	0.068
8	0.189	0.209	0.164
9	0.105	0.120	0.098
10	0.092	0.082	0.067



Mean adenylate kinase activity in CSF of 10 patients before, during and after ingestion of Hydergin. 3 CSF samples were drawn on different occasions from each of the 10 patients during the pre-treatment and treatment periods and from 6 of the patients during the post-treatment period. The calculations are therefore based on 30 determinations from each of the 2 first periods and on 26 determinations from the last period. Bars indicate ± 1SD.

CSF was drawn on 3 different occasions at about weekly intervals from each of the patients during the pretreatment period. The mean AK value of the 10 patients from the 1st sampling occasion was 0.115 units/l ± 0.027 compared to those of the 2nd and 3rd occasions being 0.111 ± 0.022 and 0.115 ± 0.032 units/l, respectively. Thus, the fact that the mean values of the 10 patients were practically the same on 3 different occasions during the 1st month without treatment contradicted the idea that there might be a spontaneous cyclic variation within the time-frame of a month of the AK activities in CSF of these patients.

Discussion. We have shown that a substantial release of AK occurs during ingestion of Hydergin by patients suffering from cerebral arteriosclerosis. The tendency towards increased activities during the treatment period compared to the pretreatment period was discernible in 8 out of 10 patients although this difference was not statistically significant. This lack of significance might be due to the limited number of patients involved in this study. When comparing the treatment period with the post-treatment period, either alone or taken together with the pre-treatment period, a statistically significant increase in AK activity exists during Hydergin treatment. This difference is of interest and indicates an increased leakage of AK through the plasma membranes of the brain cells into the extracellular fluid as represented by CSF during treatment. The explanation for this is unclear. However, it could be anticipated that the manifest presence of AK in the CSF of patients with cerebral arteriosclerosis is due to a lowered adenylate charge potential in the brain cells leading to a diminished electrochemical potential[2-5]. The effect of Hydergin might then be a further lowering of the electrochemical potential in the brain cells. This effect is most probably not exerted indirectly by a further influence on the adenylate charge potential. Instead we propose a direct action by Hydergin on the Na^+- and K^+-dependent ATPase system in the plasma membranes of the brain cells which, working in synergism with the aforementioned mechanism, results in increased leakage through the plasma membrane.

It has been reported that norepinephrine exerts activating effects on this ATPase system of brain cells[6] and the concentration of this substance in CSF was found to be increased in ischaemic brain patients[7]. This catecholamine-stimulated Na^+- and K^+-dependent ATPase activity was intensively inhibited by dihydroergot alkaloids and the inhibitory effect could be observed at as low a concentration as 1×10^{-7} M of several substances of this class including Hydergin as demonstrated in an in vitro experimental system[8,9]. Therefore, there are reasons to believe that the molecular basis for the action of Hydergin might be the same under in vivo-conditions as well. The significant lowering of the AK activity in the CSF (also in comparison with the pretreatment period) on discontinuation of the treatment with Hydergin might be explained by a rebound effect exerted by the catecholamines no longer being restrained in their action on the Na^+- and K^+-dependent ATPase system.

Thus, it seems to us that the direct action by Hydergin on the Na^+- and K^+-dependent ATPase system results in the maintenance of the intracellular ATP pool on a somewhat higher level although the resultant effect was increased leakage through the plasma membrane due to a lowered electrochemical potential.

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The effect of β-adrenergic stimulation and blockade on the efflux of PGE_1-like material from the isolated perfused rabbit heart

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Summary. Prostaglandin (PG) release was measured from the isolated perfused rabbit heart. The effects of β-adrenergic stimulation and blockade suggest that PG synthesis is regulated in part by adrenergic mechanisms.

Several authors have suggested that PGs act as modulators of hormone action and neurotransmission[2,3] Aiken and Vane[4] concluded that there was continuous PG release in the kidney which contributed to the local regulation of vascular tone. The existance of PGs in cardiac tissue is well established; however, their physiological role, and the precise mechanism underlying their effects, have received little attention. Hedqvist[3] reported that the release of catecholamines from adrenergic nerve terminals was modulated by PGE_1. The present study is concerned with the role of β-adrenergic mechanisms in the release of PGE_1 from the rabbit heart.

The rabbit heart was mounted according to Langendorff's technique and perfused with oxygenated Krebs' solution maintained at 37 °C. The hearts were allowed to stabilize for 10 min before drugs were given. The perfusate was collected during this period for 7 min to determine the resting out-put of PGE_1-like activity. The drugs adrenaline (9.5×10^{-6} M) or sotalol (7.2×10^{-2} M) were then perfused for 7 min. During this period the perfusate was collected separately and assayed for PGE_1-like activity. There was an interval of 10 min before administration of the next drug.

The PGE_1-like activity was assayed against standard PGE_1 on an isolated rat stomach strip, as described by Vane[5].

The results are summarized in the figure. The resting output was found to be 4.5 ± 0.33 ng per 7 min (n = 5). Adrenaline increased the output of PGE_1 to 7.9 ± 0.33 ng (n = 5) which was significantly different from the control