ACTION OF VASOACTIVE DRUGS ON THE CEREBRAL CIRCULATION STUDIED BY AN INFRARED METHOD

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Existing methods of studying the cerebral circulation (CC) do not completely satisfy the essential demands, due to the complexity of the function and structure of the test object [2]. The requirements of flexibility with time, informativeness, and minimal trauma were the main criteria used to evaluate the method suggested below.

EXPERIMENTAL METHOD

The method is based on the results of analysis of the spectral characteristics of the principal components of the test object, namely: the transparency of water for radiation in the infrared (IR) region to a wavelength of 1.2-1.4 μ [4], the relative transparency of skin and tissues in the near IR region [4-6], the ability of IR radiation to pass through the cranial bones [3], and differences in the reflective power of the tissues and blood in the near IR region [8, 9].

Experiments were carried out on cats anesthetized by intravenous injection of urethane (600 mg/kg) and chloralose (50 mg/kg). After division of the scalp and exposure of the parietal region of the skull a detector, consisting of an emitter and receiver of IR radiation, was fixed to the surface of the cranial bones [1]. Through the exposed cranial bone an area of the brain was continuously irradiated with IR rays and, at the same time, the intensity of reflection of IR radiation was recorded [1].

The blood pressure (BP) was measured and blood samples taken for analysis by means of a polyethylene catheter, introduced into the femoral artery. Values of pH, pCO_2, and pO_2 of the blood were determined on a model BMs3, Mark 2, microanalyzer (Radiometer, Denmark). To correct the acid-based balance of the blood when required, the animal was given an injection of the muscle relaxant listhenon (5 mg/kg intravenously every 30 min) and artificially ventilated. The intensity of IR reflection and BP were recorded continuously on an automatic writer with time constant of recording of 1-2 and 0.1-0.2 sec, respectively.

Variations of CO_2 in the blood were produced by hyperventilation or by addition of CO_2 to the inspired gas mixture. Drugs were injected into the carotid system through the lingual artery at a constant rate for 20-30 sec in a volume of 0.4-0.5 ml of physiological saline.

EXPERIMENTAL RESULTS

The effect of a change in the CO_2 concentration in the arterial blood on the change (in conventional units) of intensity of IR reflection (with the sign reversed), compared with the initial level, is shown in Fig. 1. Within the range of change of pCO_2 from 15 to 60 mm Hg, the relationship is linear in character. The complex character of interaction between IR radiation and the test object, due to the complex structure of the object, makes a correct theoretical calculation of dependence of the integral intensity of IR reflection on the intensity of CC difficult. An empirical approach is more useful as a means of obtaining the dependence of IR reflection on CC.

The results of the action of a change in CO_2 concentration in the arterial blood on CC are known, having been obtained by various methods on different animals [7, 10, 11]; they

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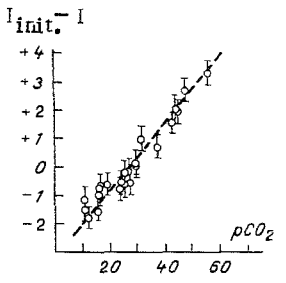

Fig. 1

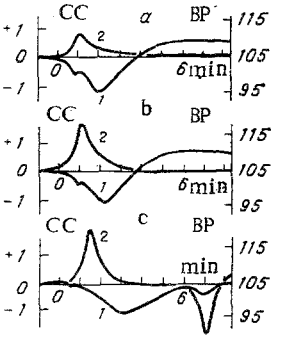

Fig. 2

Fig. 1. Effect of arterial CO_2 on intensity of reflection of IR radiation. Abscissa, pCO_2 (in mm Hg); ordinate, difference between initial and momentary value of intensity at corresponding pCO_2 level (conventional units).

Fig. 2. Time course of changes in CC (1) and BP (2) after injection of various doses of noradrenalin. Abscissa, time (in min); ordinate: left — intensity of CC (conventional units relative to initial level), right — BP (in mm Hg). Doses of noradrenalin: a) 1.2 μg/kg, b) 2.5 μg/kg, c) 5 μg/kg.

indicate that the relationship between the change in pCO_2 (within a certain range of deviations from normal) and CC is linear. In experiments on cats, this range of change of CO_2 is from 20 to 60 mm Hg.

The criterion of informativeness of the suggested method, as an indirect method of recording CC, is the character of dependence of the recorded parameter, i.e., the intensity of IR reflection, on the intensity of CC, if the transition to absolute units of CC is difficult [2]. Comparison of data in the literature and our own results indicates that the decrease in the intensity of IR reflection is a linear function of the increase in the intensity of CC. This ensures the informativeness of the method and it means that changes in the intensity of CC can be monitored (in conventional units).

The absence of trauma associated with the method is due to the fact that it is unnecessary to disturb the integrity of the skull, i.e., the main factor influencing the method of studying the state of the CC system is ruled out.

The flexibility of the method with time, as already pointed out, is determined by the time constant of recording, namely 1-2 sec. Recording the time course of changes in the intensity of CC with synchronous recording of changes in the system of the central hemodynamics enables a temporal diagram of the action of a drug to be obtained without averaging over a certain time interval (which is a disadvantage of clearance methods), a very important factor in pharmacological screening.

The action of various doses of noradrenalin (NA) on the intensity of CC was studied by the suggested method. The time course of the change in CC (in conventional units), based on the results of measurement of the intensity of IR reflection, is illustrated in Fig. 2. Doses of 1.2 and 2.5 μg/kg had a qualitatively similar action, namely they reduced CC, in agreement with the vasoconstrictor effect of NA, to the minimal value 2 min after injection of the drug, and they cause a smaller opposite effect after 4 min. A dose of 5 μg/kg shifted the minimal value of CC to 3 min, probably due to the greater contribution of BP to competition between the increase in BP and the vasoconstrictor action of NA on the cerebral vessels. The way in which CC passively follows the change in BP 6-8 min after injection of the drug may probably be due to its action on the system of the central hemodynamics.

The suggested method of studying the time course of changes in the local CC can thus be used to record complex and rapidly changing processes taking place during the action of vasoactive drugs on the CC system without disturbing the integrity of the cranial bones.

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METHOD OF DETECTING ANTIBODIES PRODUCED BY HYBRIDOMAS TO CELL NUCLEAR ENDONUCLEASES

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The study of endonucleases of cell nuclei has aroused increased interest in recent years. The use of various immunochemical and immunologic methods, and in particular, those based on hybridoma technology, may be an effective tool for such investigations.

However, considering the high heterogeneity of the nonhistone proteins of chromatin and the relatively low endonuclease content among them, the starting point for these developments must be the creation of a relatively simple but sensitive method of detection of antibodies produced against the corresponding enzymes.

In this paper one such method is described, whereby antibodies to cell nuclear endonucleases can be detected with high sensitivity and reproducibility in hybridoma culture medium.

EXPERIMENTAL METHOD

Extracts of cell nuclei containing solubilized endonucleases were obtained as follows. Nuclear residues were treated with 10 mM Tris-HCl, pH 7.4, in a volume giving a concentration of nuclear DNA of 2-4 mg/ml, and the samples were carefully suspended. Aliquots of the samples were treated with 3 volumes of 0.4 M KCl and 0.01% Triton X-100 and homogenized for 30 min in the cold. The samples were centrifuged at 12,000-15,000g for 10 min and the supernatants were used as the source of endonucleases.

Supercoiled DNA of plasmid pBR 322 was obtained by the method in [2] with minor modifications. Electrophoresis of the plasmid DNA was carried out in horizontal 1.2% agarose gels with a voltage of 1 V/cm. The conditions of photography of the gels and scanning of the negatives were described previously [1].

EXPERIMENTAL RESULTS

The method is based on the ability of endonucleases to convert closed circular DNA molecules into the open circle form and linear DNA. Since a single- or double-stranded cut is

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