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Symposium on substance P

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K. LISSÁK AND E. ENDRŐCZI

THE INHIBITORY SUBSTANCE OF THE BRAIN

In the past years publications in the literature as well as investigations by the present authors have shown that brain tissue contains a substance which may be responsible for nervous inhibitory processes. In their earlier studies the authors have stated that this substance is able to inhibit the ACh sensitivity of peripheral muscle receptors, ganglionic transmission, and, if applied locally, the excitability of the spinal cord and that of the cortex (Lissák and Endrőczi, 1949, 1955, 1956, 1957).

Concerning the vast literature I refer to the subject-matter of two International symposia (Roberts 1960, Florey 1961). In the present lecture I wish to give a picture of our own results, obtained during the last year with an extract from brain tissue, as well as with γ-amino-butyric acid (GABA), and with the biological effects of γ-amino-β-hydroxy-butyric acid (GABOB).

The extract was prepared partly from dog's brain and partly from ox brain following the method used by us in earlier experiments. Upon addition of one volume of 96% ethanol to the freshly removed tissue this mixture was homogenized and then, after addition of one more volume of ethanol and one volume of colloidal aluminium hydroxyde, centrifuged. The transparent supernatant, slightly yellow in colour, was concentrated »in vacuo« to 1/10 of its original volume, clarified with active charcoal, concentrated, then filtered, and the filtrate evaporated »in vacuo«. The residue was redissolved in 10 to 50 ml aqueous ethanol (1:1 v/v) and, after repeated treatment with active charcoal filtered and again evaporated »in vacuo«. Depending on the initial amount of the substance, the residue was dissolved in 1 to 10 ml of 50% ethanol, and then run descending on Whatman No 4 paper with a mixture of phenol-water and/or with butanol : glacial acetic acid : water (4 : 1 : 1 by volume). Different amounts of GABA and GABOB were run as tests and spotted with ninhydrin. After colour development and elution with ethanol : 0,1 N NaOH (4 : 1 v/v), estimation of the GABA content in the brain tissue was carried out photometrically with standard GABA. It should be mentioned here that a series of qualitative chemical reactions were carried out on the paper chromatograms of brain tissue extracts. Among these the for-

mation of picrate in an alkaline medium deserves to be mentioned. As it can be seen in Fig. 1, the picrate-positive area in the system butanol : acetic acid : water shows up at a position corresponding to that of GABA. I wish to mention here that in a different chromatographic system, e. g. in phenol : water, the ninhydrin-positive GABA, too, was situated similarly, relative to the picrate-positive substances in the brain tissue. The importance of this observation cannot be decided yet. However, it was the same area that, by biological testing, proved to possess inhibitory activity.

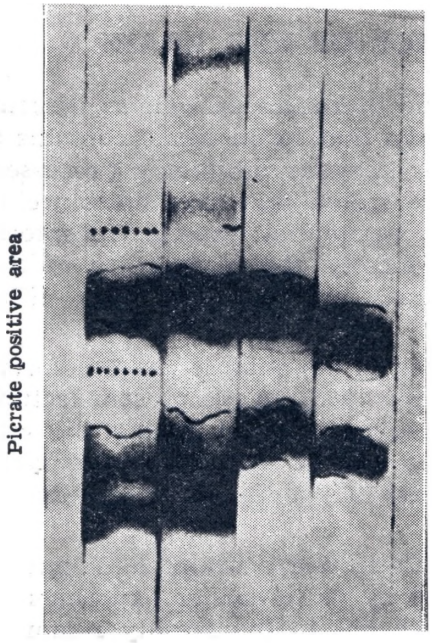


FIG. 1

Paper chromatogram of brain extract: two left stripes indicate the GABA and picrate positive area, two right ones the position of standard GABA and GABOB.

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I shall now pass on to the description of results, suggesting that the brain extract contains an inhibitory factor, but that GABA and/or GABOB can only partly be responsible for the inhibitory effect.

(a) According to observations on isolated cat ileum, the extract eluted from the paper chromatograms is able to inhibit ACh contraction at, or below, pH 7, while, at the same time, even several hundred micrograms of GABA or GABOB are ineffective. The GABA-content in the effective brain extract hardly exceeded 10—15 μg, which shows that GABA or its derivative cannot be responsible for the inhibition.

(b) When examining, on the motor cortex of the cat, the electrical threshold-stimulus for the motor reaction of a foreleg, we found that while local application of an amount of brain extract obtained from 1/3 of a dog's brain resulted in marked decrease of excitability, the same effect could not be obtained with GABA or GABOB.

(c) By means of recording the electrical activity of the cat's cortex, we examined the effects of local and systematic administration of the brain extract, and those of GABA and GABOB on strychnine and

metrazol convulsions. The recordings were made under barbiturate (Evipan-Na) or ether anaesthesia, or in succinyl-choline paralysis with artificial respiration under full consciousness. Our observations may be summarized as follows:

(1) Under barbiturate anaesthesia even high concentrations of the brain extract, GABA or GABOB administered either locally or intravenously did not result in a noteworthy effect on the electrical activity of the somatomotor cortex, recorded with bipolar silver »ball-electrodes«. Under superficial ether anaesthesia, or in succinyl-choline paralysis under full consciousness local, as well as intravenous, administration of the extract from 1/3 to 1/2 of a dog's brain resulted in decreased frequency and increased amplitude, which, however, was of a transient nature. Even large doses of GABA or GABOB failed to produce an effect worth mentioning. During metrazol convulsions produced by the intravenous administration of Tetracor, convulsive activity was considerably decreased, or, with smaller doses of Metrazol, inhibited by the extract from 1/2 to 1 dog's brain given a few seconds before Tetracor.

On the basis of Figs. 2 and 3 it can be seen that during, or immediately before, convulsive activity the intravenous administration of the extract results in a transient inhibitory effect. An attempt to produce an inhibition of a similar nature by the administration of 50—200 mg/kg GABA or GABOB also failed. Local application proved to be less effective, however, when it was used, the activity due to metrazol convulsion was often replaced by a regular synchronized activity of a frequency of 2 to 3/sec., which, without use of the extract, could only be observed very rarely and for a very short time. Even local application of GABA or GABOB failed to influence metrazol activity.

In the following part of our experiment we examined the effects of GABA, GABOB and brain extract by recording the superficial negative convulsive activity produced by strychnine. The monopolar leads were recorded in succinyl-choline paralysis under full consciousness. The amplitude of the negative discharges produced by the local application of a 1,0% strychnine solution was decreased by locally administered GABA or GABOB of 1 mg/ml concentration, which confirmed earlier observations on the subject by Purpura, Girado, Smith and Gomez, 1958. A similar result was obtained when brain extract was applied. Intravenously administered GABA or GABOB did not influence the depolarizing dendritic potentials, while the intravenous administration of brain extract not only prevented the depolarizing activity, but also had a transitory hyperpolarizing effect. Similar phenomena were also reported by Iwama and Jasper, 1957, and by Purpura and coll., 1958, although it was only in the case of a blood-brain barrier destroyed by methanol-chloroform and after the administration of large quantities of GABA that those authors observed a similar effect. From the data in Fig. 4 it may be seen that brain extract can turn the initial

depolarizing activity into hyperpolarization. The development of the mechanism is unknown. However, it is highly probable that in this experiment the abolition of the inhibitory effect of strychnine on axo-dendritic hyperpolarization plays an important rôle.

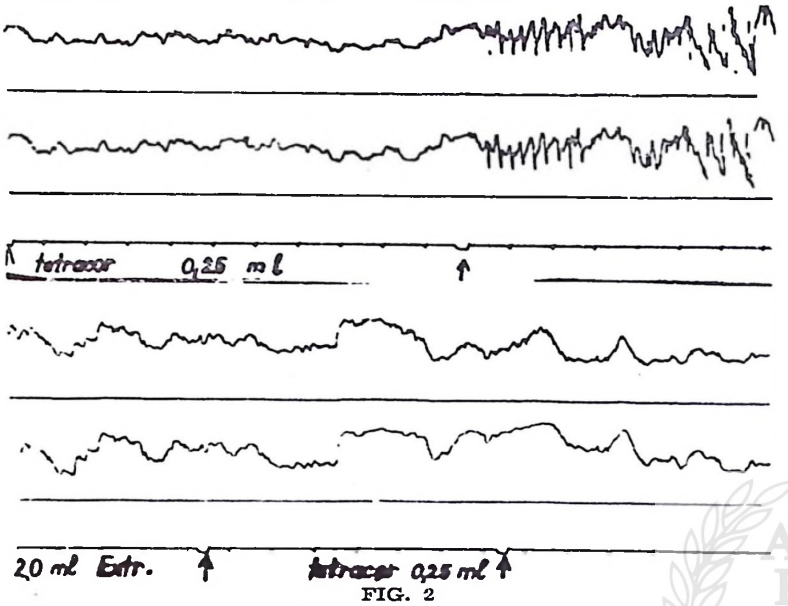


FIG. 2

Metrazol convulsion under the influence of previously administered brain extract. Upper channels : convulsive activity without brain extract. — Lower channels : blocking of the convulsive activity after intravenous administration of brain extract (at arrow).

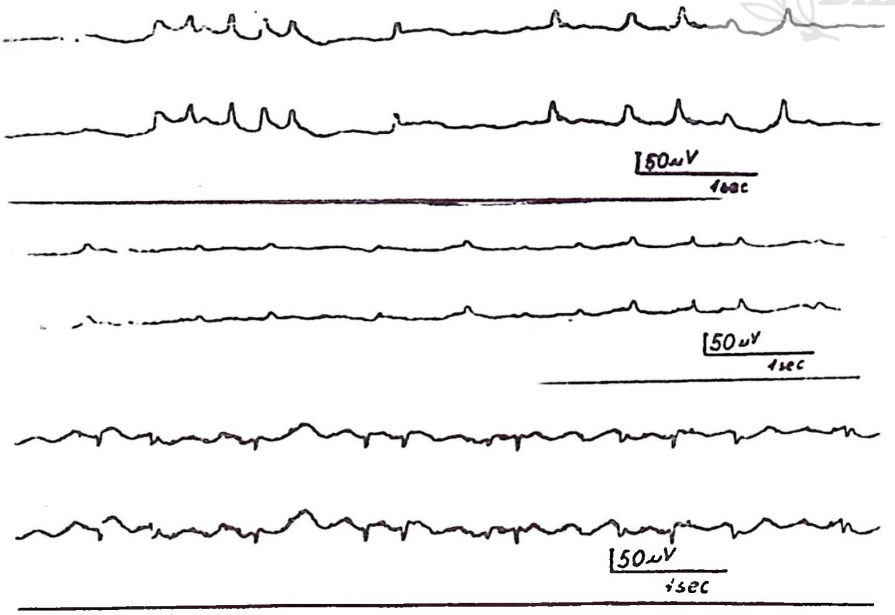


FIG. 3

The blocking effect of brain extract on convulsions induced by metrazol in the cat. Bipolar leads from the somatomotor cortex. The arrows indicate the administration of brain extract (intravenous).

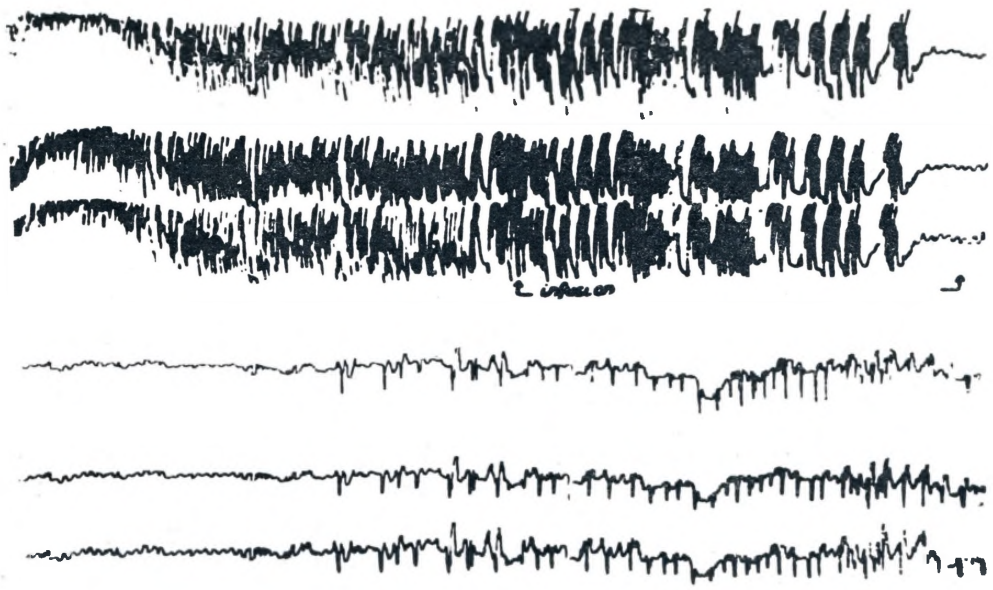


FIG. 4

Electrical activity produced by topical application of strychnine to the animal's brain. Monopolar recording : different silver-ball electrode on the motor cortex, indifferent electrode on the frontal bone. At arrow, surface negative activity was reversed by the intravenous administration of brain extract.

The action of the brain extract and that of GABA on subcortical structures were studied on the conditional reflex activity of dogs with chronically implanted microcannulas. The cannula was implanted stereotaxically, following the methods we usually employ, after the establishment of a conditioned alimentary-motor reflex. In each of the four experimental animals the cannula was localized in the reticular formation and the amount of fluid introduced varied between 0.01 and 0.05 ml. It was observed that the brain-extract strengthened the labile processes of differentiation, and shortened the duration of latency. GABA or GABOB, even in concentrations of 1—10 mg/0.1 ml, failed to produce changes in the conditional reflex processes.

In summary it may be stated that brain tissue contains a substance whose physicochemical properties, as revealed by paper chromatography, resemble those of GABA. However, the two substances differ in their effects. On the one hand, the natural inhibitory substance is more pronounced in its effects, on the other, it will act on receptors on which even high concentrations of GABA are ineffective. The basis of this disparity may be either an essential difference in structure, or the natural inhibitory substance might represent a more complex compound of GABA, which differs in permeability or has a more pronounced biological action.

In order to be able to form a final view of the mediation of inhibition it would be necessary to have a more definite knowledge of the essential properties of the inhibitory factor and the morphologic substrate through which it acts.

Summary

A number of inhibitory phenomena have been observed after administration of extracts prepared from dog's and ox' brain tissue, purified by paper chromatography. It was established that γ-aminobutyric acid (GABA) or its hydroxy-derivative (GABOB) may only partly be responsible for the inhibitory effects. The topical administration of brain extract on the motor cortex resulted in an increase of electric threshold for the motor reaction of a foreleg and a decrease of electric convulsive activity due to intravenously administered Metrazol or to local application of strychnine. The negative discharges elicited by the local strychnine application have been reduced by the brain extract but they were not influenced by GABA or GABOB. The action of brain extract and that of GABA on subcortical structures were studied on the conditioned-reflex behaviour on dogs with chronically implanted canullas. However, the administration of GABA failed to show any effect on the conditioned-reflex behaviour, local application of the brain extract in the mesencephalic reticular formation strengthened the labile processes of differentiation and shortened the duration of latency.

INHIBITIVNA SUPSTANCIJA U EKSTRAKTU MOZGA

Poslije aplikacije ekstrakata dobivenih iz mozga psa i goveda i pročišćenih kromatografijom na papiru, zapažen je niz inhibitivnih pojava. Utvrđeno je da γ-aminomaslačna kiselina (GABA) i njen hidroksilni derivat (GABOB) mogu samo djelomično biti odgovorni za te inhibitivne efekte. Lokalna aplikacija moždanog ekstrakta na motornu koru izazivala je povišavanje električkog praga za motornu reakciju prednjeg ekstremiteta, a snižavanje električke konvulzivne aktivnosti poslije davanja metrazola i. v. ili strihnina lokalno. Negativna izbijanja što ih izaziva lokalna aplikacija strihnina bila su snižena djelovanjem ekstrakta, ali ne i djelovanjem GABA ili GABOB. Djelovanje moždanog ekstrakta i GABA na supkortikalne strukture studirano je na uslovnorefleksnom ponašanju pasa s kronično implantiranim kanilama. GABA je potpuno bez efekta na uslovnorefleksno ponašanje, a lokalna aplikacija moždanog ekstrakta na retikularnu formaciju moždanog debla pojačavala je labilne procese diferencijacije i skraćivala period latencije.

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