

the administration of tryptophan, allylisopropyl-acetamide and 3-amino-1:2:4-triazole, saturation with the hematin cofactor occurs, *in vivo*, with a subsequent net increase in enzyme protein, in the form of active holoenzyme.

Studies, both *in vitro* and *in vivo*, showed that the association between apo-tryptophan pyrrolase and hematin is facilitated by tryptophan, suggesting that the concentration of the latter may determine what fraction of the existing apo-protein is catalytically active.

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1. FEIGELSON and GREENGARD (1961), *J. Biol. Chem.*, **236**, 153.

73 Extraction of a Seizure Substance (K Substance) from the Dog's Brain. T. HAYASHI and K. NAGAI (Japan).

When an electro-shock current was applied through the skull of dogs, a generalized seizure appeared, which continued 60–180 sec after the cessation of the stimulation. During these seizure, a seizure substance (K substance) was released from the motor cells which rapidly diffused into the cerebrospinal fluid. Fluid thus obtained was introduced into the c.s.f. of another dog; it produced a clonic convulsion in the receiver dog. There were, however, a few difficulties with these experiments, as success did not occur in every case. Recently, Hayashi *et al.* succeeded in all cases, if the fluid was treated with absolute methyl alcohol after reduction of its volume by a third. After centrifugation the fluid was reduced by evaporation in a 40°C water bath to 30 per cent of the original volume. This fluid contained K substance, and when it was introduced into the ventricle of the receiver dog a strong seizure was produced. The K substance could be extracted from the grey matter of the cortex, but not from the white matter.

The active agent was highly unstable in an oxygen atmosphere, but when it was kept in contact with nitrogen the activity was maintained for a long time. Purification and chemical analysis of it is now being carried out in our laboratory.

74 The Physiological Action of K Substance Extracted from Dog's Brain. T. HAYASHI and K. NAGAI (Japan).

The active agent⁽¹⁾ which was contained in the cerebrospinal fluid of dogs taken during seizure induced by electric stimulation was examined.

When a small amount of it was injected into the exposed motor cortex of a dog, a generalized seizure was produced, as when an electric stimulation was applied. For instance: (a) when concentrated, the clonic convulsion; (b) when diluted, the locomotive convulsion (walking movement);

and (c) when more diluted, a tonic movement or twitch of one limb.

When it was introduced into a ventricle, it produced seizure with latent period of 3–20 sec in the receiver dog. When it was rapidly injected through the carotid artery, a seizure appeared with latent period of 2–5 sec.

The agent had no action on the respiration and blood pressure of dogs when injected intravenously. It had no action on an excised toad's intestine, nor on an excised heart preparation.

The action of K substance was neutralized by mixing of an appropriate dose of γ -amino β -hydroxybutyric acid, which we believe was the real inhibitory transmitter of the central nervous system of higher animals. Thus we presume that the agent is the "excitatory transmitter" in the central nervous system of mammals.

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1. HAYASHI *et al.* "Extraction of a seizure substance (K substance) from dog's brain" in this congress.

75 γ -Aminobutyric Acid and Vagal Respiratory Regulation. J. A. SCHNEIDER and A. B. DRAKONTIDES (U.S.A.).

γ -aminobutyric acid (GABA), a normal brain constituent, was reported several years ago to have an inhibitory influence on crustacean stretch receptors.⁽¹⁾ Only recently, however, inhibitory or "desensitizing" effects of GABA on slowly adapting pulmonary stretch receptors of the cat were described.⁽²⁾ Since these receptors are thought to be part of the ascending arc of the Hering-Breuer Reflex, a study of the action of GABA on vagal respiratory reflexes was indicated. Simultaneous recording of whole body plethysmography and discharges from afferent pulmonary stretch receptors in cats were used to analyze respiratory characteristics during GABA injection. Tracheal occlusion responses were utilized to assess vagal respiratory function.

The study indicates that an increase in depth of the inspiratory phase and a transient elevation of the absolute lung volume coincides with the desensitizing effect of GABA on pulmonary stretch receptors. Tracheal occlusion responses, however, were not altered consistently. The relatively short duration of respiratory changes following intravenous GABA administration contrasts markedly with the prolonged inhibitory effect on pulmonary stretch receptors, indicating rapid adjustment of vagal respiratory regulation. The possible physiological significance of GABA in the regulation of respiration remains unclear.

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1. (1954), *Arch. Int. Physiol.*, **62**, 33.
2. (1960), *Amer. J. Physiol.*, **199**, 748.