thalamus itself is under the control of higher nervous centres; structures inhibiting the hypothalamic–pituitary–adrenal axis have been described particularly in the cerebral cortex, in the midbrain and in the limbic system.

Evidence for chemical transmission of impulses within the central nervous system suggests that the inhibitory effect on the secretion of ACTH exerted by the cerebral cortex might also be humorally mediated. This hypothesis has been substantiated by the data which will be presented. Intravenous injections of crude acetic acid extracts of acetone-dried powder obtained from bovine cerebral cortex significantly reduced plasma corticosterone levels in female Sprague–Dawley rats. Following gel filtration on a column of Sephadex G–25 and elution with 0.1 N ammonium acetate buffer, three different fractions inhibiting the pituitary–adrenal axis have been obtained. Their molecular weight is less than 1000; their inhibitory activity is reduced following acid or enzyme hydrolysis. The most active fraction blocks the pituitary–adrenal axis at a dose level of <50 μg.

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Effect of midbrain transections on the content of gamma-aminobutyric acid (GABA) in the cerebral cortex of the cat

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We have measured the content of GABA and glutamic acid in the cerebral cortex in cats transected (*a*) at midpontine pretrigeminal level, showing a predominantly activated e.e.g. (Batini, Moruzzi, Palestini, Rossi & Zanchetti, 1958) and (*b*) at collicular level showing a permanently synchronized e.e.g.

The transections were made with a stereotaxically oriented spatula in adult cats under halothane anaesthesia. The anaesthesia was discontinued and the cats resumed spontaneous respiration. Blood pressure was normal. The cats were killed by exsanguination 3 hr after the end of the anaesthesia and cortical samples of about 1 g were excised from both hemispheres. GABA was extracted and determined by the method of Maynert, Klingman & Kaji (1962) using one-dimensional paper chromatography. The same method of extraction was used for glutamic acid but separation was obtained by triple run chromatography. The recovery of GABA and glutamic acid added to brain homogenates was in the range of 80–90%.

TABLE 1

Level of transection:	Midpontine pretrigeminal		Collicular		
Hemisphere	Right	Left	Right	Left	
GABA (μmoles/g$\pm$S.E.)	2·06$\pm$0·1 (6)	2·09$\pm$0·1 (6)	1·41$\pm$0·04 (6)	1·33$\pm$0·09 (6)	$P<0·01$
Glutamic acid (μmoles/g$\pm$S.E.)	8·37$\pm$0·5 (4)	8·22$\pm$0·2 (4)	8·35$\pm$1·3 (3)	9·17$\pm$1·6 (3)	N.S.

Number of determinations in brackets.

The results reported in Table 1 show a statistically significant difference between the content of GABA of the cortex of the two groups of cats. No differences were found between the two hemispheres of the same cat and between the contents of glutamic acid.

These results suggest that midbrain structures influence the content of GABA of the cerebral cortex.

REFERENCES

BATINI, C., MORUZZI, G., PALESTINI, G., ROSSI, G. F. & ZANCHETTI, A. (1958). Persistent pattern of wakefulness in the pretrigeminal midpontine preparation. *Science, N.Y.*, **128**, 30–32.

MAYNERT, E. W., KLINGMAN, G. I., & KAJI, H. K. (1962). Tolerance to morphine II : Lack of effects on brain 5-hydroxytryptamine and gamma-aminobutyric acid. *J. Pharmac.*, **135**, 296–299.

The release of ^{3}H-gamma-aminobutyric acid (GABA) from rat cerebral cortex

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The status of GABA as an inhibitory transmitter in the mammalian brain remains equivocal. Although electrophysiological evidence strongly supports the suggestion that GABA is an inhibitory transmitter in the cortex (Krnjević & Schwartz, 1967), experiments performed on GABA release are less convincing. Jasper, Khan & Elliott (1966) claimed that GABA was released from the surface of the cerebral cortex and that this efflux was related to the state of activation of the brain. Efforts to reproduce these results in our laboratory have, however, been largely unsuccessful. The reason for this failure to detect appreciable changes in GABA efflux may be due to the highly efficient uptake process for GABA which is present in nervous tissue (Iversen & Neal, 1968). In order to avoid some of the difficulties of *in vivo* experiments, the release of ^{3}H-GABA from brain slices has been studied.

Slices of cerebral cortex (1 mm thick) were incubated with GABA-2,3-^{3}H (specific activity=2 c/m-mole, 0.2 μc/ml.) at 37° C in 20 ml. of oxygenated Krebs-bicarbonate Ringer containing amino-oxyacetic acid (10^{-5}M) to inhibit the metabolism of GABA. The tissue was perfused in a small vessel (volume 0.5 ml.) at a rate of 0.5 ml./min. Aliquots (0.2 ml.) of the perfusate were removed every 2 min and the radioactivity was measured by liquid scintillation counting.

The spontaneous release of ^{3}H-GABA from cerebral cortex was multiphasic with at least two major components. A rapid initial phase ($t_{\frac{1}{2}} \approx 2$ min), which presumably represented extracellular washout, was followed by a much slower release of ^{3}H-GABA ($t_{\frac{1}{2}} \approx 30$ min). Depolarization of the nerve tissue by mild electrical stimulation (rectangular pulses, 100/sec, 2 mA for 30 sec) or by Krebs medium with a high potassium concentration (40 mM) produced a striking increase in ^{3}H-GABA efflux (Fig. 1). The effect of electrical stimulation was not prevented by the absence of calcium ions in the medium. The increase in GABA efflux produced by high potassium was, however, significantly lower in the absence of calcium ($P<0.05$). The increased efflux of GABA was not a non-specific effect on the cell membrane as both these methods of stimulation failed to cause an increased release of ^{14}C-urea or ^{3}H-L-leucine from cortical slices.

The present experiments suggest that if GABA is an inhibitory transmitter in the brain it appears to use an unconventional release mechanism, because all known neurotransmitters require the presence of calcium ions for their release by electrical stimulation.