Acetylcholine release from the feline thalamus

SIR,—Thalamo-cortical relay neurons in the ventro-basal complex of the thalamus are excited by iontophoretically applied acetylcholine (Andersen & Curtis, 1964; McCance, Phillis & Westerman, 1966). Although the synaptic responses of these cells evoked by stimulation of limb nerves and the cerebral cortex are unaffected by acetylcholine antagonists, those initiated by stimulation of the mesencephalic reticular formation and brachium conjunctivum are reduced or abolished by atropine or dihydro-β-erythroidine (McCance, Phillis & Westerman, 1968). The suggestion that cholinergic pathways from the brain stem and cerebellum terminate on thalamic neurons (McCance & others, 1968) is supported by studies on the distribution of choline acetyltransferase and acetylcholinesterase in the stem and brachium conjunctivum (Feldberg & Vogt, 1948; Hebb & Silver, 1956; Shute & Lewis, 1963; Phillis, 1965).

To establish that a substance is a transmitter agent in a structure, it is desirable to demonstrate its presence in perfusates from the area during periods of physiological stimulation. For example, acetylcholine has been identified in perfusates from the cerebral cortex and caudate nucleus, and its rate of release correlated with variations in the amount of neuronal activation (Mitchell, 1963; Phillis & Chong, 1965; McLennan, 1964). The demonstration of a spontaneous release of acetylcholine from the thalamus, which can be augmented during periods of stimulation, would contribute to the confirmation of cholinergic transmission in this region of the central nervous system. A variety of modes of stimulation have been shown to cause an increase in the rate of release of acetycholine from the cerebral cortex, but as the increased release occurs in several cortical areas it is probably unrelated to the specific mode of stimulation employed. As stimulation of the reticular formation also causes an increase in cortical acetylcholine release (Kanai & Szerb, 1965; Phillis, 1968), it has been suggested that projections of the reticular arousal system are cholinergic and that the increased release observed during stimulation of peripheral structures is probably a result of activation of this system (Phillis, 1968). In the present investigation, we have shown that stimulation of various forms of input causes similar increases in release of acetylcholine from the thalamus.

Nine adult cats were used; two of these were anaesthetized with pentobarbitone sodium and in the remaining seven, anaesthesia was induced with thiopentone sodium and maintained by a gas mixture of nitrous oxide, oxygen and methoxyflurane (Penthrane, Abbott). The animals were mounted in a stereotaxic frame and after removal of the cranial vault, cortical and subcortical tissue of the left cerebral hemisphere overlying the thalamus was excised by suction to expose the hippocampal fornix and fimbria and the floor of the fourth ventricle between stereotaxic co-ordinates A7–A12 and midline to L9. A push-pull cannula (Gaddum, 1961) was inserted into the thalamus at co-ordinates A9, L5. The amounts of spontaneous release were ascertained at two depths (3 and 8 mm) below the surface of the fornix. Stimulation was employed only when the cannula tip was in the ventro-basal complex. The position of the cannula tip was verified histologically at the termination of each experiment.

Each push-pull cannula was fabricated from 27 and 18 SWG hypodermic syringe needles and mammalian physiological saline (for composition see Phillis, 1968) and was circulated through both needles by a Braun Unita II, two channel push-pull infusion pump, fitted with matching syringes. Dead space in the collecting tube was 0·1 ml, representing 10% of the standard sample volume (10 min collection period and a flow rate of 0·1 ml per min). Before sample collection, the area was perfused with a 2×10^{-5} g/ml neostigmine solution for 30

min and a similar concentration of neostigmine was present in the solutions for assay. The samples were assayed on the heart of *Tapes watlingi* (Chong & Phillis, 1965), each sample being assayed on two hearts in small (0·3 ml) Perspex baths. The inhibitory activity in the samples was abolished by the acetylcholine antagonist, benzoquinonium, or by boiling the samples briefly in an alkaline solution. The hearts were exposed to the 5-hydroxytryptamine antagonist, methysergide (UML 491) before and during assays.

Stainless steel pins were inserted into the contra- and ipsi-lateral forepaws for limb nerve stimulation, a bipolar coaxial stimulating electrode at stereotaxic co-ordinates A3, L3, D-1 was used to stimulate the reticular formation and a Grass PS 2 photostimulator provided light flashes for visual stimulation.

A spontaneous release of acetylcholine was observed in all animals at both dorsal and ventral positions in the thalamus. The rates of release were substantially reduced (50–200 pg/min) in the two animals anaesthetized with pentobarbitone sodium in comparison with the gas anaesthetized animals (100–600 pg/min). There was a marked tendency for the initial one or two samples to contain higher levels of acetycholine than those immediately following them, which suggests that implantation of a push-pull cannula may be attended by damage to nerve cells and endings with a resultant leakage of acetycholine from the fragmented tissues. After periods of stimulation, the rates of spontaneous release usually continued at an elevated level. The rates of release from both superficial and deep thalamic areas appeared to be comparable.

The three forms of stimulation all caused an increase in the rates of release of acetylcholine. In Fig. 1 are histograms illustrating the effects of visual (light flashes, A), limb, (B) and mesencephalic reticular formation (C) stimulation on the rate of release of acetylcholine from the ventro-basal complex of the

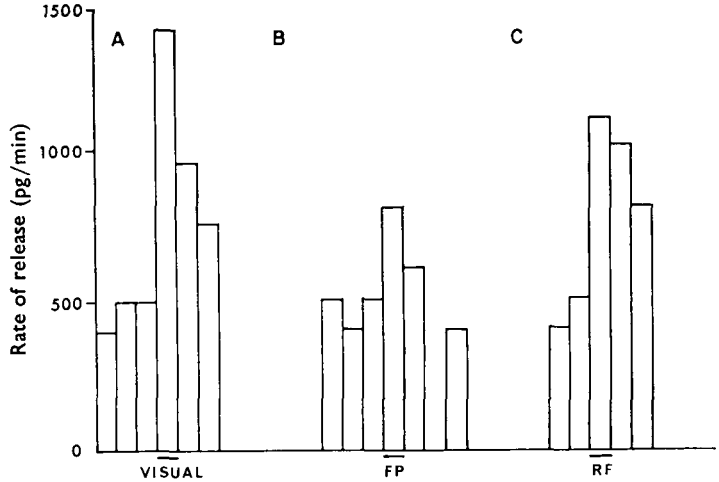

FIG. 1. A, B and C are histograms showing rate of acetycholine release (pg/min) from the ventro-basal thalamic complex of three preparations, before, during and after stimulation. Each division of abscissa represents one 10 min collection period. A: effects of visual stimulation (1/sec). B: effects of contralateral forepaw (FP) stimulation (1/sec). There was a 10 min gap between collection of the last two samples. C: effects of stimulating the mesencephalic reticular formation (RF) (2/sec). Position of the stimulating electrode was verified histologically.

thalamus. In A, the resting release of acetylcholine was 400–500 pg/min. Visual stimulation (1/sec) caused an increase to 1400 pg/min during the period of stimulation and this rate declined slowly during the subsequent collection periods. Fig. 1B shows that stimulation of the contralateral forepaw (1/sec) elevated the rate of release from 400–500 pg/min to 800 pg/min. Similarly, stimulation of the reticular formation doubled the rate of release of acetylcholine (Fig. 1C).

The effects of stimulation were frequently less marked than those demonstrated in Fig. 1 and if the initial period of stimulation resulted in a marked elevation of the rate of spontaneous release, subsequent periods of stimulation were invariably less effective. A similar observation has been made in experiments on the cerebral cortex (Phillis, 1968).

The results described support the hypothesis that acetylcholine is a synaptic transmitter in the feline thalamus (McCance & others, 1968). As the medial lemniscal pathway conveying afferent volleys from limb nerves is unlikely to be cholinergic (Andersen & Curtis, 1964; McCance & others, 1968), increased thalamic release of acetylcholine following limb nerve stimulation may be a result of activation of the reticular arousal system. A similar conclusion can be drawn from the increased acetylcholine release evoked by visual stimulation. Such conclusions are strengthened by the finding that reticular formation stimulation itself causes an increase in the rate of release and that synaptic activation of thalamic neurons by brain stem stimulation is abolished by antagonists of acetylcholine (McCance & others, 1968). The finding that acetylcholine release from the dorsal thalamus was comparable to that from the ventro-basal complex is more difficult to reconcile with studies on the distribution of acetylcholine-excited neurons in the thalamus, as they are predominantly located in the ventro-basal complex. However, acetylcholine inhibition of neurons in the dorsal thalamus has also been described (McCance & others, 1968) and the release from this area may be related to inhibitory cholinergic pathways.

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References

Andersen, P. & Curtis, D. R. (1964). *Acta physiol. scand.*, **61**, 85–99.
Chong, G. C. & Phillis, J. W. (1965). *Br. J. Pharmac. Chemother.*, **25**, 481–496.
Feldberg, W. & Vogt, M. (1948). *J. Physiol., Lond.*, **107**, 372–381.
Gaddum, J. H. (1961). *Ibid.*, **155**, 1P.
Hebb, C. O. & Silver, A. (1956). *Ibid.*, **134**, 718–728.
Kanai, T. & Szerb, J. C. (1965). *Nature, Lond.*, **205**, 80–82.
McCance, I., Phillis, J. W. & Westerman, R. A. (1966). *Ibid.*, **209**, 715–716.
McCance, I., Phillis, J. W. & Westerman, R. A. (1968). *Br. J. Pharmac. Chemother.*, **32**, 635–651.
McLennan, H. (1964). *J. Physiol., Lond.*, **174**, 152–161.
Mitchell, J. F. (1963). *Ibid.*, **165**, 98–116.
Phillis, J. W. (1965). *Experientia*, **21**, 266–268.
Phillis, J. W. (1968). *Brain Res.*, **7**, 378–389.
Phillis, J. W. & Chong, G. C. (1965). *Nature, Lond.*, **207**, 1253–1255.
Shute, C. C. D. & Lewis P. R. (1963). *Ibid.*, **199**, 1160–1164.