bution to the central actions of cigarette smoke, is however minimal, when compared with the introduction of smoke into the lungs.

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The release of amino-acids from electrically stimulated rat cerebral cortex slices

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The application of many naturally occurring amino-acids to neurones in the mammalian central nervous system has shown that some have powerful excitatory or inhibitory effects (Krnjević & Phillis, 1963; Curtis & Watkins, 1965) and this has aroused interest in the possibility that they may be involved in central synaptic transmission.

Recent experiments performed in this laboratory have shown that it is possible to demonstrate the release of gamma-aminobutyric acid (GABA) from brain slices *in vitro* when they are depolarized by electrical stimulation or by solutions containing a high concentration of potassium ion and that this release shows many of the properties associated with known neurotransmitter release processes (Mitchell, Neal & Srinivasan, 1968; Srinivasan, Neal & Mitchell, 1969). It was therefore of interest to examine, under similar conditions, the release of the other amino-acids occurring free in brain and compare this with their central actions.

In sucrose homogenates of whole brain, more than 80% of the low molecular weight amino-nitrogen is accounted for by seven amino-acids: glutamate, aspartate, GABA, glycine, serine, alanine and threonine (Whittaker, 1968). The release of these amino-acids was studied by incubating slices of cerebral cortex with the radioactively labelled compound at 37° C in 20 ml. of oxygenated Krebs-bicarbonate Ringer. The tissue was then perfused in a vessel of volume 0·5 ml. at a rate of 0·4 ml./min. The collecting vessel was changed every 2 min and 0·2 ml. aliquots of the perfusate were removed and the radioactivity measured by liquid scintillation counting. After 20 min the tissue was stimulated by electrical pulses (60/sec, 20 mA, 5 msec) for 30 sec periods at 2 min intervals for a further 20 min.

Under these conditions there was a marked increase in the efflux of glutamic acid and GABA on stimulation (significant at $P < 0{\cdot}01$); the other five amino-acids studied showed no significant increase in efflux (Table 1). Electrical stimulation also failed to increase the efflux of radioactively labelled leucine, urea and α-amino-isobutyric acid.

Electrophysiological experiments have shown that glutamate is a powerful excitant of cortical neurones, and that aspartate has weaker excitatory actions. GABA has a strong depressant action, while glycine and alanine show weaker inhibitory effects (Curtis & Watkins, 1965). It is of interest that the two amino-acids, glutamate and

TABLE 1. *Comparison of the electrically evoked increase in efflux from brain slices of seven naturally occurring amino-acids with their concentrations in whole brain and their effects when applied to cortical neurones*

Amino-acid	Concentration in brain* (μ-mole/g)	Increase in efflux on stimulation (× resting release)	Effect when applied† to nerve cells
Glutamate	7·6	2·64‡	↑ ↑
Aspartate	2·4	1·77	↑
GABA	1·9	3·11‡	↓ ↓
Glycine	1·0	1·53	↓
Serine	0·9	1·21	—
Alanine	0·6	1·06	↓
Threonine	0·2	1·44	—

↑, Excitant. ↓, Depressant. * Whittaker (1968). † Curtis & Watkins (1965). ‡ Significant at $P<0·01$.

GABA, which have the most powerful effects when applied to nerve cells, are also those which are most effectively released from brain slices by the electrical stimulation used.

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Effect of probenecid on dopamine metabolites in pigeon brain

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In many animal brains the concentrations of dopamine (DA) and of its metabolite homovanillic acid (HVA) are not very different, but in the brain of the mouse and the rat HVA is present in much smaller concentrations than DA. This suggested that in these species HVA was removed by an active transport mechanism, and this was confirmed by the finding (Sharman, 1967; Werdinius, 1967) that there is a large rise in tissue HVA after administration of probenecid, an inhibitor of transport of acidic substances from brain and kidneys. No such rise is obtained in animals in which HVA concentration is high. In pigeons, the ratio DA/HVA is 4 : 1 (Juorio & Vogt, 1967), which is intermediate between that of the two groups of mammals, and we therefore studied the effect of probenecid on the concentration of HVA and of the other acid metabolite of DA, dihydroxyphenylacetic acid (DOPAC), in this species. The brain region used was that containing the highest concentration of DA and called nucleus basalis (Juorio & Vogt, 1967). The metabolites were estimated spectrophotofluorimetrically. Probenecid was injected intramuscularly.

The amount of HVA in the nucleus basalis was $0·80 \pm 0·04$ μg/g (mean ± S.E.M.). Probenecid (200 mg/kg) increased the HVA concentration in 1·5 hr to about threefold