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Some actions of nicotine and tobacco smoke on activity of the cerebral cortex and olfactory bulb

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Small amounts of nicotine (1–4 μg/kg) injected intravenously in anaesthetized cats caused changes in cortical activity and acetylcholine release from the cerebral cortex (Armitage, Hall & Sellers, 1969). These amounts of nicotine were probably similar to those absorbed by the cigarette smoker who inhales. In the present studies a direct comparison of the actions of nicotine and tobacco smoke has been made on the brain electrical activity of the unanaesthetized cat *encéphale isolé* preparation (Bremer, 1936). The *encéphale isolé* exhibits behavioural signs of sleep and wakefulness. Actions of nicotine, cigarette smoke and carbon monoxide have been compared on preparations in the sleeping state, exhibiting a synchronized electrocorticogram consisting of slow waves and spindle activity. Samples (2 ml.) of a 25 ml. puff of smoke were introduced into the lungs at 30 sec intervals from a smoking simulator (Armitage, Hall & Heneage, 1969). This volume of smoke contains approximately 7 μg nicotine (approximately 2 μg nicotine/kg for a 3 kg cat). Samples (2 ml.) of 5% carbon monoxide were introduced into the lungs using the same simulator. Doses of nicotine and other drugs, calculated as base, were injected intravenously.

Samples (2 ml.) of smoke introduced into the lungs caused desynchronization of electrocortical activity and behavioural arousal. The electrocorticogram consisted of low voltage fast waves indicative of cortical activation, the eyes opened, and movements of the ears, jaws and vibrissae were sometimes observed. These effects were matched in the same experiment by intravenous injections of nicotine, 2 μg/kg every 30 sec. Applications of smoke or injections of nicotine required to produce these effects varied between experiments.

These changes, caused by nicotine or smoke, were not modified by pretreatment with chlorpromazine (2·0–4·0 mg/kg). Atropine (0·3 mg/kg), however, prevented the cortical activation, but not the behavioural arousal. The effects of smoke on cortical activity were generally not blocked, but only reduced by the administration of mecamylamine (2 mg/kg). In contrast, the effects of nicotine were blocked by mecamylamine, suggesting the presence in smoke of other agents capable of exerting a pharmacological response. Cigarette smoke contains approximately 5·0% carbon monoxide. Introduced into the lungs of cats pretreated with mecamylamine, 2 ml. samples of 5% carbon monoxide caused changes in cortical activity similar to those caused by smoke. The most consistent effect of smoke and carbon monoxide was the appearance of spindle bursts and low voltage fast waves.

Samples (2 ml.) of smoke applied to the nostrils caused the occurrence in the olfactory bulb of a discharge or burst of "induced" waves. This discharge was sometimes accompanied by a transient period of cortical activation. This contri-

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