Delta-9-tetrahydrocannabinol (THC) and macromolecular synthesis: mechanisms of action

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We have reported (Nahas, Desoize, Armand & Morishima, 1975) that in 10^{-5} to 10^{-4} M concentration, THC inhibits the incorporation of [^{3}H]-leucine, [^{3}H]-uridine and [^{3}H]-thymidine in cultured human lymphocytes stimulated with phytohemagglutinin (PHA). The present experiments were designed to clarify the mechanism of action of this depressant effect of THC on blastogenesis.

First, the time course of this effect of THC was studied. We observed that within 15 min after its addition, THC inhibited the incorporation of three precursors of protein, RNA and DNA: leucine, uridine and thymidine. This data indicates that THC does not exert its inhibitory effect specifically on the synthesis of a single macromolecule; such a type of specific inhibition is characterized by a lag in the synthesis of the macromolecules that are not primarily affected by the inhibition. In the present experiments, cell synthesis of all three macromolecules was inhibited at the same time.

In a second series of experiments we measured the effect of THC on uptake of leucine, uridine and thymidine into the intracellular pool. This uptake was significantly inhibited by the drug. This inhibition of precursor uptake in the cellular pool can explain the impairment of macromolecular synthesis.

Pool formation of precursors may be inhibited by three mechanisms which were next studied. Inhibition of transport enzymes, increased cellular 'leaking', or inhibition of ATP synthesis. Cellular 'leaking' was tested by incubating for 2 h lymphocytes with [^{14}C]-aminoisobutyric acid (a substance which is transported into the cell, but not incorporated into macromolecules); the cells were then washed, incubated for 30 min with THC and centrifuged; radioactivity was measured in the supernatant; it was not different from that obtained in control preparations. Effect of THC on cellular energy production was tested by: (a) measuring effect of THC on lymphocyte anaerobic glycolysis (glucose consumption and lactate production): it was not affected by the drug; (b) measuring cellular ATP concentration: there was no significant decrease compared with control cultures.

It would therefore appear that THC exerts its inhibitory effect on macromolecular synthesis to a greater extent by interfering with the transport enzymes of the plasma membrane. THC is known to bind on the plasma membrane (Chari-Bitron, 1971). At the level of the plasma membrane THC might act directly by altering its physico-chemical characteristics (Seeman, 1972). THC would therefore exert its inhibitory effect on cell anabolism during every stage of the cell cycle.

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Is the gastric antisecretory property of clonidine in rats of central origin?

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It seems now established that even though clonidine is ineffective (Boissier, Giudicelli, Larno & Fichelle, 1970) or even stimulates the gastric secretion of anaesthetized rats as it does in pentagastrin-stimulated Heidenhain pouch dogs (Curwain & Endersby, 1974), it possesses powerful gastric antisecretory properties in conscious rats (Walz & Van Zwieten, 1970) and in man (Kaess & Von Mikulicz-Radecki, 1971). This report describes the effects of clonidine in the 4 h-pylorus ligated rat and in basal or stimulated gastric secretion in chronic fistula rats in order to verify the hypothesis that this action is probably of central origin.

Dose response curves were established in male Sprague–Dawley rats (250–300 g) either by the 4 h-pylorus ligated Shay rat preparation or in 2 × 2 h basal secretion tests in chronic gastric fistula rats (Pascaud, Errard & Blouin, 1974), some of the latter bearing stereotaxically implanted micro-cannula in the lateral ventricle of the brain. On rats with gastric fistulae, the

effect of clonidine (30 μg/kg s.c.) was determined on acid secretion stimulated by continuous i.v. infusions (2 ml/h) in a tail vein of histamine (1 mg kg^{-1} h^{-1}), pentagastrin (6 μg kg^{-1} h^{-1}), 2 deoxy-*d*-glucose (2 DG, 25 mg kg^{-1} h^{-1}) and methacholine (0.6 mg kg^{-1} h^{-1}). Total acidity was automatically titrated at pH = 8.5 (ATS_1, Radiometer).

Gastric acid output decreased markedly after clonidine in both pylorus ligated and chronic fistula rats under basal conditions. The corresponding ED_{50}s were 27 (15–48) μg/kg, i.p., and 13.6 (5.4–34) μg/kg, s.c. respectively. In contrast the ED_{50} was 5.8 (1.6–21) μg/kg when clonidine was administered in the lateral ventricle of the brain. Furthermore clonidine (30 μg/kg s.c.) produced a very significant ($P < 0.01$) and long lasting (>3 h) inhibition when maximal acid responses were obtained with histamine, pentagastrin or 2 DG infusion. On the contrary no inhibition was seen on the secretion stimulated by methacholine.

Thus clonidine used in the same dose range as that described in dogs for its cardiovascular activities possesses powerful gastric antisecretory properties in basal and stimulated acid secretion of conscious rats. Considering first the marked inhibition obtained on the 2 DG stimulation and the lack of activity on the methacholine, and second the very low ED_{50} registered when clonidine was administered in the lateral ventricle of the brain, it seems very likely that its gastric antisecretory property is of central origin in rats. Work is now in progress to determine more precisely the nature of the central neurone receptors involved in this activity.

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Effects of *p*-chloro-β-phenylethylamine on the uptake and release of putative amine neurotransmitters in rat brain

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The monoamine *p*-chloro-β-phenylethylamine (PCPE), a metabolite of *p*-chlorophenylalanine (PCPA) has been reported by Koe & Weissman (1966) to cause a decrease in brain noradrenaline (NA) and 5-hydroxytryptamine (5-HT) levels following its injection intraperitoneally into rats. Our findings in littermate male Wistar rats, using the assay procedure of Martin & Ansell (1973), are in agreement with this. NA levels were 74.8 ± 3.5% and 5-HT levels 74.7 ± 4.8% of controls (mean ± s.e. mean, $n = 6$) 15 min after PCPE, 40 mg/kg i.p. In the same animals we found no significant change in dopamine (DA) levels from controls.

We have now investigated the effects of PCPE on uptake and release of ^{3}H-labelled DA, NA and 5-HT *in vitro* using methods previously described (Raiteri, Angelini & Levi, 1974; Raiteri, Levi & Federico, 1974). Crude synaptosomal P_2 fractions (Gray & Whittaker, 1962) prepared from rat hypothalamus (NA studies) or striatum (DA and 5-HT studies) were used, and all incubation and superfusion media contained nialamide (1.25×10^{-5} M).

PCPE was a stronger inhibitor of 5-HT and NA uptake than of DA uptake ($IC_{50} = 2.2 \times 10^{-6}$ M, 5.0×10^{-6} M and 1.5×10^{-5} M respectively) and, as shown in Figure 1, showed marked differences in its effect on the release of the three transmitters, being a particularly strong stimulator of 5-HT release. Simultaneous experiments with β-phenylethylamine demonstrate that addition of the *p*-chloro substituent on β-phenylethylamine has little effect on NA release, but has a dramatic influence on 5-HT and DA release, increasing the former and decreasing the latter.

This compound appears to be a useful analogue for studying structure-activity relationships in the effects of phenylethylamine derivatives on the transport of amine neurotransmitters *in vivo* and *in vitro*, although to maintain substantial levels *in vivo* for long periods