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The evidence of the release of prostaglandin-like material from rabbit kidney and guinea-pig lung by (−)-*trans*- Δ^9-tetrahydrocannabinol

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Injection of (−)-*trans*-Δ^9-tetrahydrocannabinol (THC) through the renal artery caused a decrease in perfusion pressure and an increase in urine produced by the isolated perfused rabbit kidney. Both effects of THC are inhibited by the prior addition of aspirin to the perfusion medium. THC also induced a dose-dependent increase in perfusion pressure on the isolated perfused lung of guinea-pig and the effluent from the lung produced a contraction on the isolated continuously superfused rat stomach fundus strip. These effects are prevented by the pretreatment of the lung with aspirin which inhibits the production of prostaglandins (PG) and SC 19220 which inhibits the pharmacological effects of PG.

The pharmacological effects of both resin of cannabis and (−)-*trans*- Δ^9-tetrahydrocannabinol (THC) have been extensively studied on *in vitro* and *in vivo* preparations within last few years. Bose, Vijayvargiya & others (1963) have shown that cannabis resin produces an inhibitory effect on the respiratory movements of anaesthetized dogs and a relaxation of the intestinal smooth muscle of rabbit and uterus of rat. It also antagonizes the spasmogenic effects of cholinergic agents, histamine, barium chloride and vasopressin in these isolated preparations. Layman & Milton (1971) have recently observed that THC and cannabidiol do not inactivate the cholinergic sites of guinea-pig ileum and inhibit twitch responses of transmurally stimulated guinea-pig ileum. They have shown that these compounds inhibit the response of guinea-pig ileum to acetylcholine and histamine and also cause a reduction in the spontaneous release of acetylcholine. A detailed *in vitro* study has recently been made by Gascon & Pérès (1973). These authors have reported that THC does not have agonistic activity on the vas deferens, the phrenic nerve-diaphragm and ileum preparations. They have also shown that THC produces transitory potentiation followed by inhibition of the acetylcholine-induced contraction of the isolated vas deferens as well as potentiation of the contractile activity of noradrenaline. Moreover THC does not modify the contraction induced by nerve stimulation although it abolishes the effects of anti-cholinesterase inhibitors in this preparation.

The effect of THC on the isolated perfused organs has not previously been studied. We here report an investigation of the release of PG-like material(s) from the isolated perfused rabbit kidney and isolated guinea-pig lung under the influence of THC.

MATERIALS AND METHODS

The experiments were performed on the isolated perfused rabbit kidney or guinea-pig lung from adult animals of either sex.

The kidneys from rabbits anaesthetized with sodium pentobarbitone (30 mg kg^{-1}) were isolated according to Gündogan & Türker (1974) and perfused through the renal

artery by a peristaltic pump delivering 17 ml min^{-1} throughout the experiments. Perfusion fluid was Krebs-Henseleit solution aerated with 5% CO_2 in oxygen and warmed (37°). Perfusion pressure was measured by a pressure transducer (Statham P 23 Dc). Urine drops were recorded simultaneously from the cannulated ureter by a magnetic tipper.

The lungs from guinea-pigs anaesthetized with sodium pentobarbitone (35 mg kg^{-1}) were isolated and perfused through the pulmonary artery according to Bakhle, Reynard & Vane (1969). The perfusion flow was 10 ml min^{-1} throughout the experiments. Perfusion pressure was recorded by a pressure transducer (Statham P 23 Dc). The outflow was continuously superfused over a rat stomach fundus strip (RSF) prepared according to Vane (1957). RSF strips were first isolated and superfused with Krebs solution. They were subjected to 2 to 4 g initial tension. Contractions were recorded by an isometric transducer (Grass FT. 03). A steady state baseline was established during 1 h, and thereafter the strips were superfused with lung effluent.

The kidney was first perfused with normal Krebs solution for 2 or 3 h until a steady state of perfusion pressure and urine excretion was established. The lungs were allowed to perfuse for 30 min before the effluent was superfused over the RSF strips.

In the first series of experiments THC was injected through the renal artery at different doses and the changes in perfusion pressure and urine volumes were measured. In each experiment the solvent of THC was also injected by the same route. Injections were made at 20 min invervals. After measuring the control dose-response, aspirin ($1{\cdot}6 \times 10^{-5}$ M) was added to the perfusion medium as described by Gilmore, Vane & Wyllie (1968) and the experimental procedure was repeated.

In a second series of experiments the dose-response curves for PGE_2 and THC were recorded from the RSF strip continuously superfused with Krebs solution and these were repeated when the strips were superfused with the lung effluent. THC was next given through the pulmonary artery and the changes in both perfusion pressure and RSF were simultaneously recorded. Then aspirin ($1{\cdot}6 \times 10^{-5}$ M) was added to the perfusion fluid and the procedure repeated.

In a third series of experiments, SC 19220 (l-acetyl-2-(8-chloro-10,11-dihydrobenz-[b, f][1,4]-oxazepine-10-carbonyl)-hydrazine) which is a specific competitive blocker of PGE_2 (Sanner, 1969), was used instead of aspirin.

In some experiments atropine (0·5 μg ml^{-1}), phenoxybenzamine (0·5 μg ml^{-1}) and mepyramine (1 μg ml^{-1}) were added to the perfusion medium when the effect of THC was tested.

The changes in perfusion pressure were measured as mm Hg for kidney and mm H_2O for lung. The change in urine flow was expressed as % increase compared with the control value; measurements were taken 2 to 5 min before and after injection of THC.

A 0·1% emulsion of THC was prepared in saline containing 1% Tween-20. Since the original THC solution obtained from NIMH was in 20% ethanol, the THC emulsion contained 0·5% ethanol. The solvent used in control experiments contained 1% Tween-20 and 0·5% ethanol in saline. Further dilutions were made with saline.

The following drugs were used: (−)-*trans*-Δ^9-tetrahydrocannabinol, NIMH, Maryland, U.S.A.; PGE_2, Upjohn, U.S.A.; SC 19220, Searle, U.S.A.; Lysine aspirin, Bayer, Germany.

The results were evaluated using Student's *t*-test.

RESULTS

A single injection of THC into the renal artery of the isolated kidney induced a dose-dependent fall in perfusion pressure as well as an increase in urine volume (Fig. 1). An equal volume of solvent produced a small and transitory depression in perfusion pressure without affecting urine volume. These effects were inhibited after addition of aspirin to the perfusion fluid at concentrations of 10^{-6} to 10^{-5} M. The log dose-response curve of THC taken from 15 experiments is shown in Fig. 2.

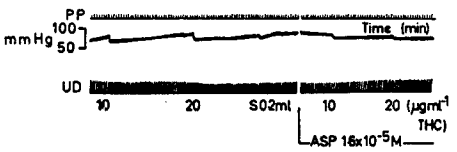

FIG. 1. Isolated perfused kidney of rabbit. Upper tracing (PP) shows perfusion pressure, lower tracing (UD) represents urine drops from cannulated ureter. THC was injected via the renal artery. (S) indicates solvent, (ASP) aspirin.

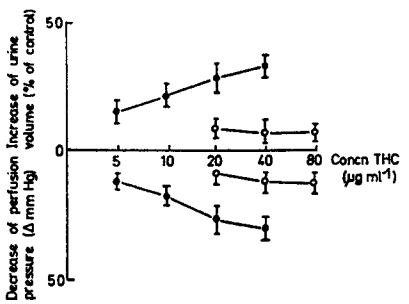

FIG. 2. Dose-response curve of THC in the isolated perfused rabbit kidney before and after addition of aspirin to the perfusion medium. Each point represents the mean value of 15 experiments. Vertical bars shows s.e.m.

Single injections of THC into the pulmonary artery of the isolated guinea-pig lung produced a dose-dependent rise in perfusion pressure while the solvent given through the same route induced a fall. When the effluent was superfused over the RSF strip, it caused a contraction which was related to the dose of THC. When aspirin $1{\cdot}6 \times 10^{-5}$ M or SC 19220 5 μg ml^{-1} was added to the perfusion fluid, there was significant inhibition of the contraction of RSF as well as inhibition of the rise of perfusion pressure (Figs 3, 4). Aspirin did not change the effect of PGE_2 directly applied to RSF strips. Addition of atropine (0·5 μg ml^{-1}), phenoxybenzamine (0·5 μg ml^{-1}) and mepyramine (1 μg ml^{-1}) did not abolish the effect of THC on perfusion pressure or the effect of THC-injected lung effluent on RSF strips. The amount of PG-like material released by THC from the perfused lung was estimated by comparing the response of the RSF to perfusate and to PGE. The results were:

Concentration of THC (μg ml^{-1})	Amount of PG-like material (ng equivalent to PGE_2)
Normal lung effluent	0
5	0·73 ± 0·14 mean ± s.e.m.
10	1·83 ± 0·35 n = 6

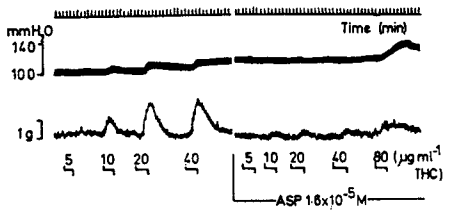

FIG. 3. Record from the isolated perfused lung of guinea-pig. Upper tracing shows perfusion pressure, lower tracing represents the isometric contractions of rat stomach fundus strip superfused with lung effluent. THC given through the pulmonary artery raised the perfusion pressure and the effluent caused a contraction in the RSF strip. Both effects are prevented by aspirin (ASP).

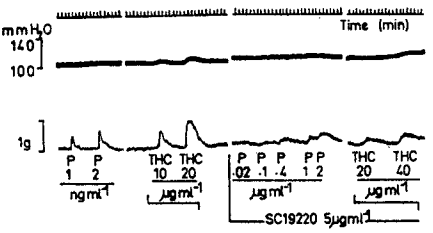

FIG. 4. Record from the isolated perfused lung of guinea-pig. (1) The effect of adding PGE_2 (P) to the lung effluent as it superfuses the RSF, (2) the effect of THC injected via the pulmonary artery, (3) the direct effect of PGE_2 on RSF superfused with the lung effluent containing SC 19220, (4) the effect of THC in the presence of SC 19220.

DISCUSSION

The results show that THC has diuretic and vasodilator effects on the isolated perfused rabbit kidney. Both effects are inhibited by aspirin pretreatment but not by atropine, phenoxybenzamine or mepyramine. These findings show that THC acts on the kidney by causing a release or formation of an endogenous substance which can be prevented by aspirin which is a potent inhibitor of the biosynthesis of prostaglandins (Vane, 1971). When PGE_2 was given through the renal artery it caused a fall in perfusion pressure and an increase in urine flow. These findings were taken as evidence of the possibility that THC can cause an increase of the biosynthesis of prostaglandins in the kidney, especially as the E and A series of prostaglandins are reported as the normal humoral mediators of the kidney by many authors (Lee, Covino & others, 1965; Lee, Crowshaw & others, 1967; Fülgraff, Brandenbusch & Meiforth, 1972). The dose-response curves of THC in the perfused kidney (Fig. 2) show that the antagonism by aspirin is not competitive.

The experiments on the perfused guinea-pig lung support the hypothesis that THC causes release of PG-like material. Injection of THC into the pulmonary artery caused a rise in perfusion pressure, and the effluent produced a dose-related contraction on the RSF which is known to be extremely sensitive to PGs (Coceani & Wolfe, 1966).

THC does not have a direct effect on the RSF. The identity of the active agent as PG was supported by the finding that addition of aspirin or the specific competitive inhibitor, SC 19220, (Sanner, 1969) into the perfusing medium significantly inhibited the response of RSF to the effluent from the THC-injected lung.

That pulmonary vasoconstriction produced by THC could be blocked by aspirin and SC 19220 but not by phenoxybenzamine and mepyramine strongly suggests that this effect is mediated by the release of PG-like substance(s) rather than by catecholamines and histamine.

Diuretic and renal vasodilatator effects of THC are not new findings. Ames (1958) reported an increase of urine output in volunteers after ingestion of cannabis. Barry, Peach & Kubena (1970) found that THC in rats increased the urine flow twofold over 8 h. In addition, the typical eye-redness in man after taking either marihuana or THC by any route is due to vasodilatation.

There have been some recent studies on the interactions of THC and PGs. Burstein & Raz (1972) reported that THC inhibits *in vitro* synthesis of PGE_2 from its precursor arachidonic acid. However, the inhibitory effect of THC was less potent than indomethacin. In fibroblast cultures THC antagonized the effects of PGE_1 and noradrenaline both of which elevate cyclic AMP levels (Kelly & Butcher, 1973). Our results show the evidence of the release of PG-like substances from the investigated tissues following THC administration and thus are not in accordance with the inhibition of either the synthesis or the effects of prostaglandins.

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