Mean arterial blood pressure (MAP) and catecholamines (NE+E) expressed as ng/g wet wt of tissue, or µg/organ, following renal denervation or sham-denervation in spontaneously hypertensive rats

Age Weeks	n	MAP mmHg	Kidney ng/g	Small intestine ng/g	Adrenal µg/organ
Denervated kidneys					
6	5	125±10*	3.4±1.0*	39.0±17.8	6.99±0.89
8	5	144±9*	8.7±2.3*	76.2±20.7*	9.98±0.63
10	4	146±6*	25.5±7.2*	63.4±8.4*	11.50±3.72
14	5	159±4*	43.8±18.7*	48.3±12.2*	12.28±3.66
17	5	181±12	35.3±10.6	63.2±13.9*	8.21±1.60
Sham-denervated kidneys					
6	5	151±2	239.4±150.8	254.1±132.5	5.26±0.90
8	5	164±4	59.2±7.0	143.0±24.6	8.76±1.88
10	4	163±2	79.2±21.1	165.1±42.6	12.92±1.86*
14	4	178±5	94.9±18.3	133.6±31.7	15.25±2.56*
17	4	193±13	81.8±25.8	187.0±42.9	6.74±1.36

* Value significantly different from that measured in the sham-denervated control group (Student's t-test), and from adrenal catecholamines content at 6 weeks of age, when a statistical significance for the mean effect was reached (analysis of variance), i.e. in group-2 ($F = 5.65$), but not in group-1 ($F = 0.95$).

surgical stress than normal Wistar rats (unpublished personal data).

NE+E disappeared almost completely from the denervated kidney the week after surgery. These low levels of renal NE+E at 6 weeks, and to some extent at 8 weeks of age, demonstrate the efficiency of our procedure on the renal biosynthesis of catecholamines, and lend further support to a major role played by catecholamines in the development of hypertension. The duration of renal denervation was apparently short since the NE+E content began to rise again 3 weeks after the surgical procedure. There was an interesting parallel between the mean blood pressure and the renal NE+E contents in rats with denervated kidneys: the NE+E content returned towards values found in sham-denervated animals as hypertension developed. From our data, we have no information about the mechanism through which renal catecholamines depletion delays the development of hypertension in SHR.

The adrenal catecholamines content showed no statistically significant difference between denervated and sham-denervated groups, but an age related increase from 6 to 14 weeks, that was statistically significant at 10 and 14 weeks of age in group-2. We cannot draw any conclusion from these differences, but one may wonder whether an increase of the adrenal secretion rate of catecholamines could not play a synergic role in the development of high blood pressure in SHR's.

The renal denervation did not affect significantly the catecholamine levels in the spleen (results not shown) but did so in the small intestine. However the evolution of arterial pressure appeared to correlate best with the renal levels of catecholamines since there was a significant difference in both arterial pressure and kidneys catecholamines levels from 6 to 14 weeks. On the other hand, the difference in catecholamines content persisted in the small intestine even at 17 weeks, at which time hypertension was fully developed.

In conclusion, our data show that bilateral denervation of kidneys in SHR is associated with a striking reduction of the renal NE+E content. Although the NE+E concentration in the small intestine is also reduced, the delay in blood pressure increase observed in SHR following bilateral renal denervation is best explained by a temporary destruction of renal noradrenergic fibres.

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Interactions of Δ^1-tetrahydrocannabinol with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography[1]

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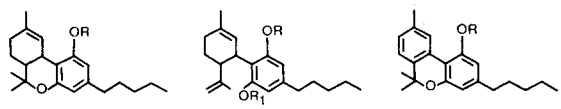
Summary. Oral administration to man of 20 mg Δ^1-tetrahydrocannabinol (THC) together with placebo or 40 mg cannabinol (CBN) or 40 mg cannabidiol (CBD) gave evidence of a possible limited interaction of THC with CBN but not with CBD as indicated by average plasma THC levels. Peak CBD and CBN concentrations were similar to that of THC, viz. about 5-8 ng/ml.

The major pharmacological activity of cannabis is due to Δ^1-tetrahydrocannabinol (THC) and its 7-hydroxylated metabolite[2]. 2 other major constituents, cannabidiol (CBD) and cannabinol (CBN) show no or little psychoactive effect, respectively. Thus, 20-400 mg CBN or 20-100 mg CBD given p.o. to man, or 5-30 mg CBD i.v. produced no characteristic effects of cannabis[3]. Perez-Reyes et al. similarly concluded that CBD i.v. had no effect and CBN i.v. was $^1/_{10}$ as potent as THC in man[4].

Studies in animals have indicated interactions between the major cannabinoids[5-7]. CBD inhibited the mixed function oxidase system in vitro[6,7], although in man Lemberger et al.[7] found no pharmacokinetic interaction between CBD and secobarbital. When CBD was smoked together with



Δ¹-THC
I R=H
II R=TMS

CBD
III R, R1=H
IV R, R1=TMS

CBN
V R=H
VI R=TMS

Figure 1. Structures of Δ^1-THC (**I**), CBD (**III**) and CBN (**V**). The deuterium label of the internal standards is located in the terminal methyl groups of the 5 carbon side chains. For mass fragmentography the compounds were converted to the corresponding trimethylsilyl (TMS) derivatives (**II, IV, VI**).

THC it was found that CBD had no influence on the acceleration of heart rate caused by THC[7]. However, the euphorogenic action and the effects of THC in some performance tests tended to be attenuated by the coadministration of CBD[7]. That CBD diminishes several of the effects of oral THC in man was also shown earlier[8]. Hollister and Gillespie investigated the interactions of CBD and CBN with orally administered THC[5]. No influence was observed with CBN but CBD tended to delay and prolong the effect of THC. In another study CBN tended to potentiate some of the effects of THC in man, but not others[9].

In the present study we investigated the interaction in man of orally administered CBD and CBN with THC as revealed by plasma levels. Since no assay for CBD or CBN has been described a mass fragmentographic method was developed.

Assay. The assay for CBD and CBN in human plasma is analogous to that published for THC previously[10-12]. The cannabinoid is extracted from blood plasma and then purified by liquid chromatography on Sephadex LH-20. The purified cannabinoid is silylated and assayed by mass fragmentography using the deuterated analogue as internal standard. The deuterium labeled internal standards: Δ^1-THC-d_3, CBD-d_3 and CBN-d_3 were described earlier[10,12].

To a 5.0-ml plasma sample, 100 µl of an internal standard solution – containing circa 10 ng deuterated standard – was added. The sample was extracted and purified on a 1×40 cm Sephadex LH-20 column as described for THC[10]. The elution volumes were for: Δ^1-THC, 24–30 ml; CBD, 28–33 ml; and CBN, 30–35 ml. The purified cannabinoid extract was trimethyl silylated with BSTFA[12].

An LKB 2091-051 gas chromatograph-mass spectrometer fitted with an 2091-711 multiple ion detector was used for the quantitative analysis. Ionizing potential, 50 eV; trap current, 50 µA; flash heater, 260 °C; ion source, 270 °C. Separations were made on a 2.1 m×2 mm inner diameter 3% JXR/Gas Chrom Q column at 230°C. The base peaks in the mass spectra of the silylated compounds (fig. 1) were selected for monitoring: m/z 390 and 393 for CBD and CBD-d_3 $[M-68]^{+\cdot}$, retention time 3.5 min; m/z 386 and 389 for THC and THC-d_3 $[M]^{+\cdot}$, retention time 4.7 min; and m/z 367 and 370 for CBN and CBN-d_3 $[M-15]^+$, retention time 6.5 min.

Subjects. 12 men, ranging in age from 18 to 40 years, were volunteer subjects. All had previous experience with marijuana. Each received 3 treatments at weekly intervals in a balanced cross-over sequence: 20 mg of THC and placebo; 20 mg of THC and 40 mg of CBD; 20 mg of THC and 40 mg of CBN. The drugs in ethanolic solution were laid onto a chocolate cookie with the solvent evaporated under nitrogen, and the cookies were then eaten. After each dose, blood samples were drawn as shown in figure 2 and the plasma analyzed for cannabinoids[11].

Results and discussion. The mean plasma level (±SD) from 20 mg oral THC shows a peak of about 5 ng/ml at 1–2 h declining to 1 ng/ml after 10 h (fig. 2A). Co-administration of 40 mg CBD did not notably influence the pharmacokinetics of oral THC (fig. 2B). Thus, the earlier discussed attenuation of some THC effects by CBD can probably not be attributed to a change in the disposition of THC itself but rather to interaction at the receptor level. To what extent the metabolite 7-hydroxy-Δ^1-THC contributes to the activity of oral THC is still unknown, but pronounced changes in its kinetics could perhaps be caused by CBD.

The concurrent administration of CBN and THC gave a similar profile to that for THC itself (fig. 2C). There is, however, a tendency, although not a statistically significant one, towards slightly higher THC levels during the first 6 h.

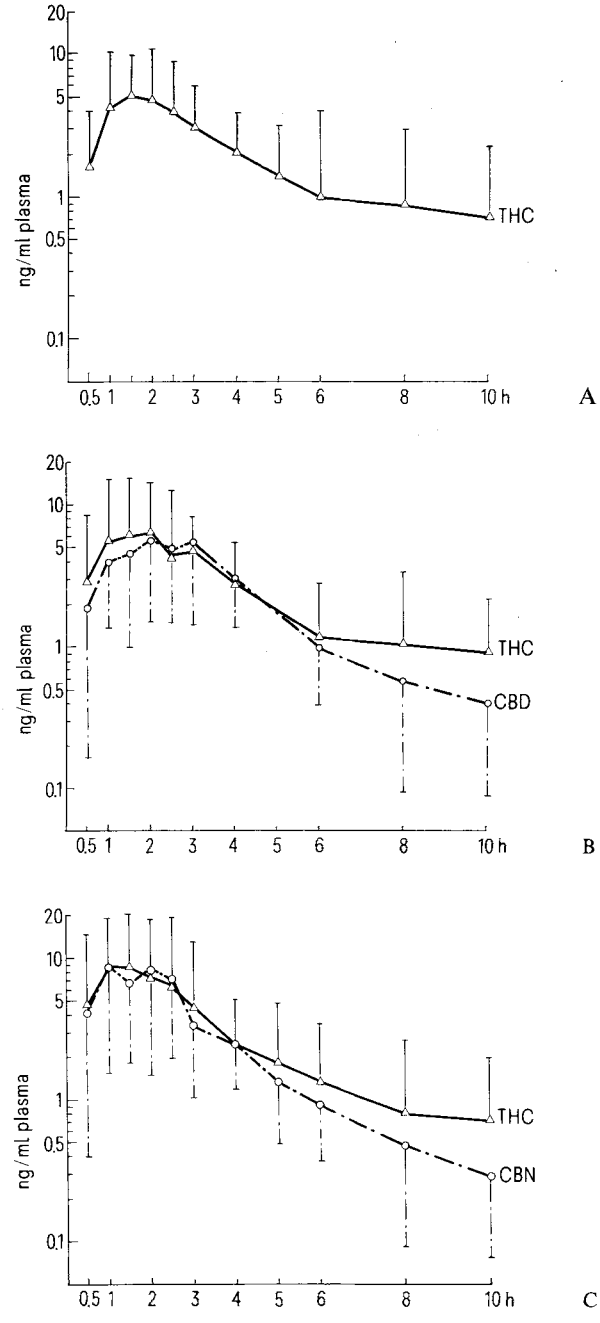


Figure 2. Average plasma levels (±SD, n=12) after oral administration of *A* 20 mg THC, *B* 20 mg THC and 40 mg CBD, and *C* 20 mg THC and 40 mg CBN.

This could, presumably, explain a slight potentiating effect of THC in combination with CBN found in one study[9].
Our results also show that oral administration of 40 mg of CBD or CBN gives average plasma levels similar to 20 mg THC. Both CBD and CBN levels decrease more rapidly than THC after 6 h. Whether this is due to differences in absorption, distribution or elimination is unclear. The limited pharmacokinetic data available[13], obtained with radiolabeled CBD and CBN, suggest that the latter 2 compounds may have shorter half-lives than THC in man.
The mass fragmentographic assay described here for CBD and CBN is as sensitive as that described for THC, is specific and can be used down to levels of about 0.1 ng/ml for CBN and about 30 pg/ml for CBD.

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Prostaglandin-mediated contractile effect of impromidine and dimaprit in the isolated rat stomach fundus[1]

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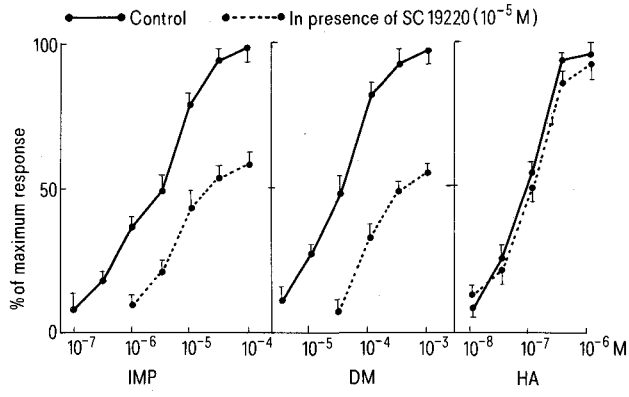
Summary. Impromidine and dimaprit, two newly-synthesized histamine H_2-receptor agonists, have been shown to produce a prostaglandin-mediated contraction in the isolated rat stomach fundus.

The presence of histamine (HA) receptors has been demonstrated in the rat stomach fundus (RSF). The predominant contractile effect of HA on this tissue is mediated through H_1-receptors, while the relaxing effect of the amine is through H_2-receptors[2]. 4-Methyl HA, a pure H_2-receptor agonist, at concentrations of 10^{-8}–10^{-6} M, produces a relaxation in the RSF which can be inhibited by metiamide. However, impromidine (IMP) and dimaprit (DM), all newly-synthetized pure HA H_2-agonists[3,4], have recently been shown to produce a contractile effect in the rat stomach fundus, and this effect could be inhibited by a prostaglandin (PG) receptor blocker and PG-biosynthesis inhibitor. The present study deals with the details of this observation.

Materials and methods. The experiments were performed on isolated RSF strips from adult rats of both sexes, weighing between 200 and 300 g[5]. Strips were continuously superfused with Krebs' solution, aerated with 5% CO_2 in O_2 and maintained at 37 °C, at 10 ml/min flow rate. In some experiments the strips were mounted in an isolated organ bath (10 ml) containing Krebs' solution. Isotonic contractions of the strips were recorded on a kymograph by means of a frontal lever with 12-fold magnification. After an equilibration period of 1 h, the dose-response curves were obtained with IMP, DM and HA before and after SC 19220, a specific PG-receptor blocker[6], was added to the bathing medium. A similar series of experiments was carried out using equipotent concentrations of IMP, DM and HA in the presence of aspirin (ASA), a well-known inhibitor of PG-biosynthesis[7]. Other agonists, 5-HT, angiotensin II and acetylcholine were also tested. The results were expressed as percent of maximum response and statistically evaluated using Student's t-test.

Results. IMP and HA produced a dose-dependent contraction when added to the bathing medium. A similar contraction was obtained with DM at a relatively higher concentration (up to 10^{-4} M). Neither mepyramine (10^{-6} M) nor metiamide (10^{-5} M) altered the responses induced by IMP and DM. Mepyramine, however, at the same concentration competitively inhibited the contractile effect of HA. Atropin (10^{-6} M) and methysergide (10^{-6} M) were also found not to change the contractile response to both H_2-agonists and HA in rat stomach fundus strips. Prior addition of SC 19220 to the bathing medium at a concentration of 10^{-5} M



Dose-response curves of impromidine (IMP), dimaprit (DM) and histamine (HA) before and after SC 19220. A significant inhibition was obtained in the responses to IMP and DM but not to HA. Each point represents the mean value of 10 experiments. Vertical bars show SE of mean.