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Pharmacological properties of tetrahydropapaveroline

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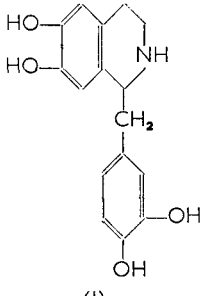
Tetrahydropapaveroline (THP) exerts β-sympathomimetic effects similar to those of isoprenaline. On guinea-pig isolated atria, THP elicits positive inotropic and chronotropic activities which are not abolished by previous reserpinization of the animals; on isolated mammalian heart these effects are associated with an increase in coronary flow. In the dog, THP increases myocardial contractile force and rate, elicits a hypotensive effect and stimulates respiratory activity in normal and reserpinized animals; when injected intra-arterially the drug causes vasodilatation. All the effects are prevented by the β-adrenergic blocking agents propranolol, dichloroisoprenaline and pronethalol. Structure-activity relationships between tetrahydroisoquinoline derivatives and their open-ring phenylethylamine congeners, which are closely related to sympathomimetic drugs, are discussed.

THE pharmacological properties of tetrahydropapaveroline (THP), described by Laidlaw (1910), have been reviewed recently by Holtz, Stock & Westermann (1964) and by Santi, Bruni, Luciani & others, (1964) and Fassina, Tóth & Santi (1965). THP produces β-sympathomimetic effects similar to those of isoprenaline.

Holtz, Stock & Westermann (1963) obtained experimental evidence that THP may be a naturally occurring substance, since it is formed *in vitro* by guinea-pig liver mitochondria by condensation of dopamine, the precursor of adrenaline, with its primary product of oxidative deamination, dihydroxyphenylacetic aldehyde, but the *in vivo* formation of THP has not yet been demonstrated. It therefore seemed worthwhile comparing the pharmacological behaviour of THP, papaverine and isoprenaline.

Chemically, tetrahydropapaveroline is 1-benzyl-3′,4′,6,7-tetrahydroxy-1,2,3,4-tetrahydroisoquinoline (I).



(I)

Experimental

Acute toxicity was assessed in mice of either sex, 20–22 g, by intraperitoneal injection of THP suspended in 5% acacia gum. The LD50 was determined according to Weil (1952) by using 6 doses and 5 animals per dose; the animals were observed for 5 days after drug administration.

Spasmolytic activity was examined on isolated guinea-pig and rabbit ileum suspended in a 30 ml bath containing Tyrode solution at 37°

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bubbled with air. THP was compared with papaverine against acetylcholine (10^{-7}), histamine (10^{-7}) and barium chloride (10^{-4})-induced contractions of the guinea-pig ileum. A dose cycle of 10 min was used, the spasmolytic drugs being allowed to act for 3 min. The effect of THP and isoprenaline on spontaneous contractions and tone was tested on rabbit duodenum.

Effect on myocardial activity. Guinea-pig isolated atria were suspended vertically in a 30 ml bath containing oxygenated Webb (1950) solution at 29°.

Reserpinized atria were obtained by pretreating the animals with reserpine 2 mg/kg i.p. daily for two days.

Guinea-pig isolated hearts were perfused with the conventional Langendorff technique. Coronary flow rates were measured by a flowmeter.

The effects on myocardium *in vivo* were examined in open chest dogs by means of the strain gauge technique described by Boniface, Brodie & Walton (1953). Some experiments were made on dogs reserpinized according to Paasonen & Krayer (1958).

Vasodilator effects on the hind limb of the dog were obtained by recording flow through the femoral artery with a Shipley & Wilson (1951) rotameter. The effects on vascular smooth muscle in the rabbit isolated ear perfused according to the technique of Krawkow & Pissemski (Ther, 1949) were also examined.

Blood pressure and respiration effects were assessed in dogs and cats anaesthetized with pentobarbitone sodium, 30 mg/kg i.v., and guinea-pigs anaesthetized with urethane, 1–1·2 g/kg. Carotid blood pressure was recorded with a mercury manometer or a Sanborn pressure transducer model 267 B. THP was administered intravenously at doses ranging from 0·005 to 5 mg/kg. In the cat, effects on the nictitating membrane stimulated through the right cervical sympathetic chain by rectangular pulses (0·2 msec duration at 10 pulses/sec, for 10 sec, every 10 min) were also examined.

Short-circuit current and potential difference changes of the isolated frog skin were measured according to the technique of Ussing & Zerahn (1951).

Oxidative phosphorylation. The effect of THP, isoprenaline and papaverine on oxidative phosphorylation was tested using rat liver mitochondria, isolated according to Hogeboom (1955). Measurement of oxygen uptake was made by the conventional Warburg technique at 30°. Inorganic phosphate was determined by the procedure of Fiske & Subbarow (1925); mitochondrial protein was measured as described by Gornall, Bardawill & David (1949).

DRUGS

Drugs used were: tetrahydropapaveroline hydrochloride (Inst. Pharm. Chem. Padua), papaverine hydrochloride (Hoffmann La Roche), isoprenaline sulphate (Abbott), adrenaline bitartrate (Recordati), acetylcholine hydrobromide (Farmitalia), bradykinin (Sandoz), histamine

hydrochloride (Hoffmann La Roche), propranolol hydrochloride, pronethalol hydrocloride (I.C.I.), reserpine (CIBA), heparin (Vister), dichloroisoprenaline (SKF).

Results

ACUTE TOXICITY

The intraperitoneal LD50 of THP in mice was 703 mg/kg (fiducial limits 586 and 843 mg/kg). With doses up to 200 mg/kg the animals showed no untoward effects; larger doses caused marked sedation, with eyelid ptosis, bradypnoea followed, sometimes, by apnoea and death. The LD50 of papaverine given i.p. in rats was 89 mg/kg (fiducial limits 18 and 101 mg/kg).

SPASMOLYTIC ACTIVITY

On the guinea-pig ileum the spasmolytic activity of THP was less than that of papaverine. Concentrations of 1×10^{-6} THP produced a 10–50% inhibition of contractions caused by acetylcholine, histamine or barium chloride, but with increasing concentrations of THP there was a decrease in the amount of inhibition produced (Fig. 1). Unlike

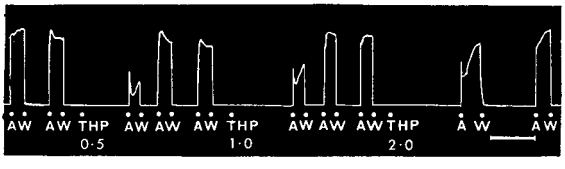

FIG. 1. Isolated guinea-pig ileum. Antispasmodic effect of tetrahydropapaveroline (THP, $\times 10^{-5}$) with a decrease in effect after repeated increasing doses. A, acetylcholine 2×10^{-7}. W, wash. Time scale = 3 min.

papaverine, THP caused a similar inhibition of the rapid and the tonic phases of the contractions to the agonist drugs.

On the rabbit duodenum THP reduced both tone and spontaneous contractions. The effects of 2×10^{-7} THP were comparable to those obtained with 4×10^{-8} isoprenaline and in each case the inhibition was reduced by propranolol 1×10^{-6} (Fig. 2). However this result is difficult to interpret because propranolol itself inhibited the tone and spontaneous contractions of the intestine.

FIG. 2. Isolated rabbit duodenum. (a) effect of isoprenaline (ISO, 4×10^{-8}) before and after propranolol (P, 10^{-6}). (b) effect of tetrahydropapaveroline (THP, 2×10^{-7}) before and after propranolol. W, wash. Time scale = 3 min.

EFFECTS ON MYOCARDIAL ACTIVITY

THP in concentrations of 5×10^{-8} to 5×10^{-7} increased both rate and force of contraction of guinea-pig atria (Fig. 3). THP was approxi-

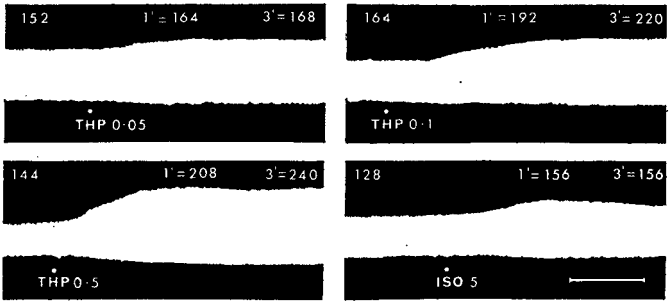

FIG. 3. Isolated guinea-pig atria. Stimulant effects of tetrahydropapaverolinc (THP, $\times 10^{-6}$) and isoprenaline (ISO, $\times 10^{-9}$). Time scale = 1 min.

mately 20 times less potent than isoprenaline. Atria from animals pretreated with reserpine were unresponsive to tyramine but more responsive to THP, which was effective at concentrations as low as 5×10^{-9}. Furthermore, pronethalol (1×10^{-7}), dichloroisoprenaline (1×10^{-7} g/ml) or propranolol (1×10^{-7} g/ml) blocked the effect of THP and of isoprenaline.

In the guinea-pig perfused heart, THP at concentrations from 2 to 8×10^{-8} increased amplitude and rate of contraction, with a simultaneous increase of coronary flow (Fig. 4).

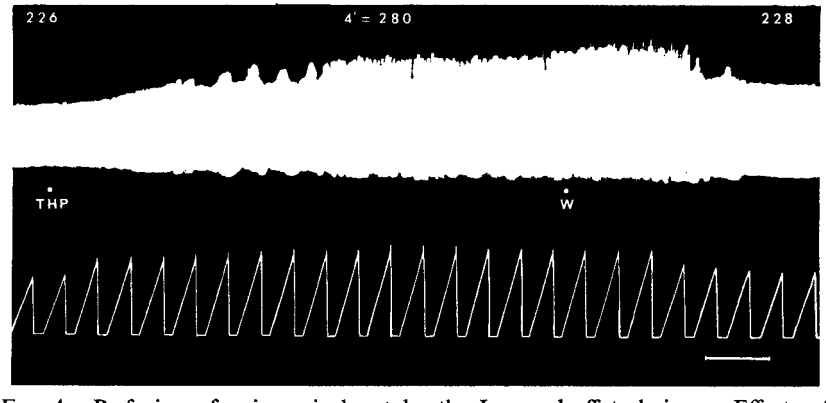

FIG. 4. Perfusion of guinea-pig heart by the Langendorff technique. Effects of tetrahydropapaveroline (THP, 5×10^{-8}) on contractile activity and coronary flow. Time scale = 1 min.

The intravenous administration of 5 μg/kg of THP to the dog increased the force (70–80%) and the rate (15–20%) of the cardiac contraction. THP was about 50 times less active than isoprenaline and 200 times more active than papaverine. Propranolol (0·25 mg/kg) abolished the increase in contractile force caused by isoprenaline (0·05 μg/kg), THP

(5 μg/kg), or papaverine (1 mg/kg) but did not prevent the fall in blood pressure produced by papaverine. In dogs pretreated with reserpine, THP and papaverine maintained their activity, whereas tyramine was ineffective.

EFFECTS ON FLOW THROUGH THE FEMORAL ARTERY AND RABBIT ISOLATED EAR

THP injected intra-arterially in the dog at doses ranging from 3 to 15 μg/kg increased the femoral blood flow by 25–100%. The effect was about 50 times less than that of isoprenaline, but twice that of papaverine. Propranolol (0·25 mg/kg i.v.) blocked the increase in flow caused by THP or isoprenaline but failed to block the effect of papaverine.

The vasoconstrictor effect induced by continuous perfusion of the rabbit ear with 5×10^{-8} adrenaline was abolished by the injection into the lateral cannula of THP, 500 μg; this effect lasted 1–2 min. The effect of papaverine was about the same but more persistent (2–4 min).

EFFECT ON BLOOD PRESSURE AND RESPIRATION

In agreement with the findings of Laidlaw (1910) THP gave a clear hypotensive effect in dogs, cats, guinea-pigs and rabbits, at doses ranging from 0·005 to 5 mg/kg i.v. A significant stimulation of the respiration accompanying the fall of blood pressure, was also confirmed. Dichloroisoprenaline (5 mg/kg), pronethalol (3 mg/kg) or propranolol (0·25 mg/kg) blocked the hypotensive effect of THP (Fig. 5) but in similar experiments the hypotension caused by papaverine was unaffected.

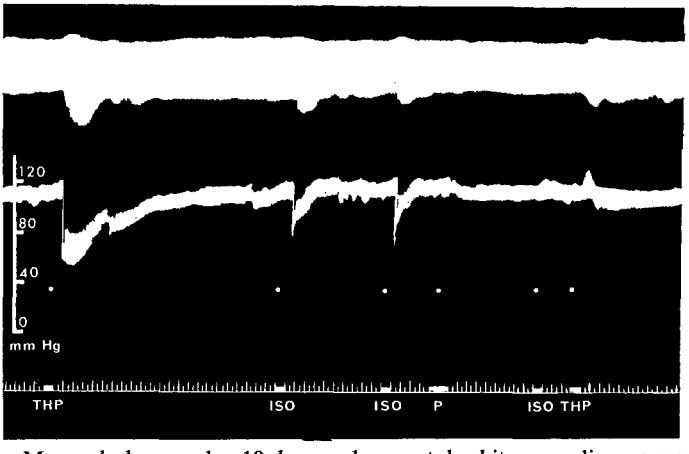

FIG. 5. Mongrel dog, male, 10 kg, under pentobarbitone sodium anaesthesia. Effects of tetrahydropapaveroline (THP, 0·05 mg/kg) and isoprenaline (ISO, 0·1 μg/kg) before and after propranolol (P, 0·25 mg/kg) on carotid blood pressure and on respiration. Time in min.

SUPERIOR CERVICAL GANGLION-NICTITATING MEMBRANE

THP in doses causing a pronounced fall in blood pressure (5 mg/kg i.v.) failed to impair the responses of the cat nictitating membrane to electrical stimulation of the preganglionic cervical sympathetic nerves.

SHORT-CIRCUIT CURRENT AND POTENTIAL IN THE ISOLATED FROG SKIN

THP and isoprenaline ($1{\cdot}5 \times 10^{-4}$M) induced a significant increase of the short-circuit current but the slight increase of the potential difference was not statistically significant. Papaverine ($2{\cdot}7 \times 10^{-4}$M) on the contrary elicits a statistically significant fall in both the short-circuit current and the potential difference (Table 1). The effects of both

TABLE 1. FROG SKIN POTENTIAL AND SHORT-CIRCUIT CURRENT RESPONSE TO TETRAHYDROPAPAVEROLINE, ISOPRENALINE AND PAPAVERINE

Number of skins	Treatment	Skin potential difference ΔmV	%	P	Short circuit current difference ΔμA	%	P	Time (min) Potential	Current
	Tetrahydropapaveroline								
10	none	0±6	—	—	0±17	—	—	60	60
5	$4{\cdot}2 \times 10^{-5}$M	+13±5	(30)	>0·20*	+ 70±20	(26)	<0·05*	18± 5	24± 5
5	$1{\cdot}5 \times 10^{-4}$M	+ 7±3	(12)	>0·40	+ 68± 6	(37)	<0·02	19±11	37±10
3	$3{\cdot}0 \times 10^{-4}$M	+10±3	(17)	>0·20	+107±22	(67)	<0·05	37± 9	53± 7
	Isoprenaline								
5	none	0±6	—	—	0±12	—	—	60	60
3	$1{\cdot}5 \times 10^{-4}$M	+4±1	(11)	>0·50	+66±15	(34)	<0·05	3±2	17±6
	Papaverine								
7	none	0±3	—	—	0±7	—	—	60	60
7	$2{\cdot}7 \times 10^{-4}$M	−24±4	(57)	<0·01	−77±20	(68)	<0·01	128±38	138±38

Skin of *Rana esculenta* was mounted in a lucite chamber and attached to a short circuiting apparatus equivalent to that described by Ussing & Zerahn (1951). Short circuit current and skin potential are expressed as differences as well as percent variations from pretreatment values. Times reported are those at which response reached its maximum. Control skin values were measured for 60 min. Means ± s.e. * P = significance of difference from untreated controls. The drugs were added to the Ringer solution bathing the two sides of the skin.

THP and isoprenaline were prevented by 10^{-3}M pronethalol and 10^{-4}M propranolol.

EFFECTS ON OXIDATIVE PHOSPHORYLATION

THP or isoprenaline failed to inhibit the uptake of oxygen or phosphate using either glutamate or succinate as substrate whereas papaverine 0·1 mM abolished both oxygen and phosphate uptake with glutamate as substrate, without affecting the oxidation of succinate (Santi, Ferrari & Contessa, 1964).

Discussion

The findings suggest that the effects of THP are of a β-sympathomimetic nature. This statement is supported mainly by the close similarities of the effects displayed by isoprenaline and THP and by the antagonism of these drugs by β-adrenergic blocking agents.

These conclusions agree with the previous findings of Holtz & others (1964) and are consistent with the results of the investigations on molecular interaction on lipolysis *in vitro*, clearly indicating an affinity of THP for the same receptor system as isoprenaline (Fassina & others, 1965). Quantitatively, THP appears to be from 1/10 to 1/50 as active as isoprenaline, depending on the method of assessment. However, on isolated frog skin THP was as active as isoprenaline in increasing the short-circuit current.

In spite of their structural similarity, remarkable pharmacological

differences are evident between THP and papaverine; the β-sympathomimetic mechanism is not involved in the hypotensive effects of papaverine, as demonstrated by the results obtained with propranolol and dichloroisoprenaline. Furthermore the difference in behaviour between papaverine and THP is evident in the experiments on oxidative phosphorylation, isolated frog skin and spasmolytic action.

It seems that both the hydrogenated isoquinoline ring and the hydroxy groups are essential for the sympathomimetic activity of THP, because the unsaturated compound papaveroline, 3,4-dihydropapaveroline and tetrahydropapaverine are devoid of any sympathomimetic activity (Tóth, Ferrari, Contessa & Santi, 1966). The study of these isoquinoline compounds on lipolysis *in vitro* leads to the same conclusion (Fassina & others, 1965).

In the extensive investigation of Hjort, deBeer & Fassett (1938, 1940) and Fassett & Hjort (1938), the close similarity of activity of the β-phenethylamines and their cyclized 1,2,3,4-tetrahydroisoquinoline derivatives is pointed out. In the light of these conclusions, THP, which has hydroxy-groups in positions 6 and 7, might be considered a cyclized derivative of epinine. This could account for the direct sympathomimetic activity of THP, even if it does not explain its selective β-sympathomimetic activity.

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