EFFECTS OF 1,3,5-TRIHYDROXYBENZENE AND 2,4,6-
TRIHYDROXY-1-PROPIOPHENONE ON THE
SMOOTH MUSCLE ORGANS

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Although 1,2,3-trihydroxybenzene (pyrogallol) is known to inhibit the activity of
catechol-o-methyltransferase (COMT) (1, 2), its clinical application is limited because
of toxic effects such as formation of methemoglobin and degeneration of the kidney and
liver (3). Pyrogallol has been useful only as an experimental tool to modify the metabolic
process of exogenous as well as endogenous catecholamines. The present experiments
were facilitated by the facts that 1,3,5-trihydroxybenzene (phloroglucinol, THB) and 2,
4, 6-trihydroxy-1-propiophenone (THPP) showed the definite spasmolytic action in ex-
perimental animals (4, 5) and that these compounds relieved the spastic disorders of the
urinary tract, bile duct and uterus in patients (6). Based on the experimental findings
that the mode of spasmolytic action is neither atropine-like, anti-histamine-like, nor pa-
paverine-like, Cahen (4) has suggested a direct musculotropic action of these compounds.
Hattori et al. (7) have shown that trihydroxybenzenes inhibit slightly to markedly COMT
activity in vitro. The acute, subacute and chronic toxicity tests of THB in experimental
animals confirmed that it is of low toxicity and wide margin of safety (8). In the present
experiments the pharmacodynamic effects of THB and THPP on the smooth muscle or-
gans were tested taking into consideration whether the spasmolytic actions of trihydroxy-
benzenes are correlated with COMT inhibition and subsequent potentiation of the action
of catecholamines.

METHODS

1,3,5-Trihydroxybenzene (THB) was supplied from Nippon Roussel Pharmaceutical
Co., Tokyo and 2,4,6-trihydroxy-1-propiophenone (THPP) was from Eisai Pharmaceu-
tical Co., Tokyo. Pyrogallol was used as control.

1. Cardiovascular system

Carotid arterial blood pressure of amobarbital sodium-anesthetized dog and rabbit
was recorded on the smoked paper via a mercury manometer. Respiratory movement
was recorded via a tracheal cannula and a tambour on the same paper.

The movement of isolated rabbit atria was recorded at 30°C with a spring lever.
2. Urinary tract

Isolated urinary bladder: The urinary bladder was isolated from mongrel dog (weighing 10 to 15 kg) after killing, and it was filled with Ringer-Locke solution through a glass cannula inserted via the ostium urethrae. Then, the bladder was immersed in the oxygenated Ringer-Locke solution at 33°C and the intravesical pressure was adjusted to obtain a maximal contractile response to 10^-6 g/ml of acetylcholine. The intravesical pressure was recorded on the smoked paper through the cannula and tambour. The rhythmic contraction of the isolated guinea-pig or rat bladder suspending in the oxygenated Ringer-Locke at 33°C was recorded isotonically on the smoked paper.

Urinary bladder in situ: According to Edge (9) and Oyaizu (10), anesthetized dog (amobarbital sodium 40 mg/kg, i.p.) was laparotomized in the midline. In most cases, the rectum, some parts of large and small intestines were eviscerated. The warm physiological saline was filled in the intravesical cavity through a Nélaton catheter inserted via the urethra. The volume of saline solution in the cavity was adjusted to obtain a maximal rhythmic contraction. The intravesical pressure was recorded via a mechano-electric transducer on the pen-writing oscillograph. The hypogastric and pelvic nerves were exposed and immersed in paraffin pool at 37°C. The cut peripheral end of either nerve was stimulated with square wave, 20/sec, 1 msec, submaximal or maximal, for 5 seconds every 3 minutes. The square wave was delivered from a Nihon Koden Type MSE-3 electronic stimulator.

Isolated ureter: Guinea-pigs weighing 200 to 300 g, rabbits weighing 1.5 to 2.0 kg and dogs 5 to 10 kg were sacrificed, and the ureter was isolated. The rhythmic contraction of the ureteral strip, suspended in the oxygenated Ringer-Locke solution at 28°C, was recorded isotonically on the smoked paper.

Ureter in situ: After laparotomy of anesthetized dog, the unilateral ureter was exposed, then a polyethylene tube was inserted to the ureteral lumen from an opening made surgically at the proximal end, and another tube was from the ostium ureteris. Warm saline was perfused through the ureteral lumen at a constant pressure to give about 40 drops/min. In another series of experiments, the intraureteral pressure was recorded on a pen-writing oscillograph via a polyethylene tube filled with saline and a mechano-electric transducer.

3. Gallbladder and bile duct

Isolated gallbladder: Guinea-pigs weighing about 200 g and dogs weighing 5 to 10 kg were sacrificed, then the gallbladder was isolated. Suspending in the oxygenated Ringer-Locke solution at 37°C the rhythmic contraction of guinea-pig gallbladder was recorded on the smoked paper. With the dog gallbladder, the intravesical pressure was recorded on the smoked paper.

Perfusion of the bile duct in situ: Following the method of Boissier and Chivot (11), amobarbital sodium anesthetized dogs were laparotomized in the midline, and the gallbladder, bile duct and duodenum were exposed. A polyethylene tube was inserted to the bile duct from an opening made surgically at the proximal part of the duct, and another tube was from the duodenal papilla. Warm saline from the Mariotte flask was perfused
through the bile duct. A bubble flowmeter was set between the flask and the proximal tube, and the flow rate was adjusted to about 0.5 ml/min.

4. Isolated uterus, vas deferens and intestine

Adult female rats or guinea-pigs were sacrificed by head amputation at the estrus and the whole uterine horns were removed. The uterine strip was suspended in the oxygenated Ringer-Locke or De Jalon solution at 37°C and the rhythmic contraction was recorded isotonically on the smoked paper. The vas deferens was isolated from adult male rats and the contraction was recorded similarly. The movement of isolated rat or rabbit intestine was also recorded in a similar way.

RESULTS

1. Cardiovascular actions

The intravenous injection of pyrogallol at doses of 1 to 10 mg/kg did not affect, or only slightly and transiently elevated the blood pressure of anesthetized dog. But the doses significantly potentiated and prolonged the pressor response of 1 µg/kg of adrenaline or noradrenaline (Fig. 1). Respiratory movements were not affected. On the other hand, the intravenous dose of THB 1 to 10 mg/kg affected neither blood pressure level nor respiratory movements. The injection of THPP at doses above 10 mg/kg produced a slight and transient elevation of blood pressure level without affecting spontaneous respiratory movements. Prior injection of THB or THPP above 10 mg/kg potentiated the pressor response to catecholamines. However, the potentiating action of THB was less than that of pyrogallol. The injection of 1,2,4-trihydroxybenzene (1, 2, 4-THB) at doses of 1 to 10 mg/kg produced not only the pressor effect but also the potentiation of catecholamine response. The injection of 1 or 2 mg/kg of 1,3,5-trimethoxybenzene (TMB) lowered the

![Graph](https://example.com/graph1.png)

Fig. 1. The effect of trihydroxybenzene (THB) on the pressor response to adrenaline (Ad) in anesthetized dog.
blood pressure level slightly and decreased the catecholamine pressor response transiently. The actions of THB and THPP in anesthetized rabbits were not different from those in anesthetized dogs.

The amplitude of contraction of isolated rabbit atria was potentiated slightly and transiently after application of THB in concentrations above $2 \times 10^{-4}$. THPP in concentrations of $10^{-5}$ and $10^{-3}$ produced a slight but progressive increase in amplitude as well as rate of atrial contraction. However, THPP at the concentration of $10^{-4}$ resulted in a progressive depression of atrial rhythm, and finally in a cardiac arrest. THB and THPP in concentrations of $10^{-6}$ and $10^{-3}$ potentiated the positive inotropic and chronotropic effects of exogenously administered catecholamines, but these effects were significantly less than those of pyrogallol.

2. Effects on urinary tract

a. Urinary bladder: Although the application of THB or THPP in concentrations below $10^{-4}$ did not affect the intravesical pressure of the isolated dog urinary bladder, the rise of pressure due to acetylcholine $10^{-6}$ was significantly decreased by either agent. The anti-acetylcholine action of TMB was more potent. The intravesical pressure elevated by BaCl$_2$ $10^{-3}$ was decreased by papaverine $10^{-6}$, and TMB $10^{-4}$ but not by THB or THPP $10^{-1}$.

The rhythmic contraction of isolated rat bladder was transiently depressed by adrenaline or noradrenaline in concentrations above $5 \times 10^{-4}$. The depressing effect of catecholamines was intensified by pretreatment with THB, 1,2,4-THB and pyrogallol. The potency was in the reverse order of above description. The contraction of isolated guinea-pig bladder was somewhat irregular and depressed by adrenaline or noradrenaline in concentrations above $10^{-5}$. The depressing effect was also intensified by THPP, THB and pyrogallol in concentrations above $10^{-6}$. Again pyrogallol was most effective. The acetylcholine-induced tonic contraction was partially or completely blocked by THPP $10^{-2}$ to $10^{-4}$. Pyrogallol was effective at the concentration of $10^{-6}$.

The intravesical pressure of the urinary bladder of anesthetized dog was not affected by the intravenous doses of 10 to 50 mg/kg of THB or THPP, and 10 mg/kg of pyrogallol or TMB. The rise of intravesical pressure in response to maximal stimuli on the hypogastric nerve was not affected, or rather slightly potentiated, by the intravenous injection of hexamethonium 5 mg/kg or atropine 1 mg/kg. Tolazoline 5 mg/kg produced a complete block of hypogastric stimuli following an initial transient facilitation. The injection of bretylium 5 mg/kg resulted in a similar but more sustained block. THB 10 to 50 mg/kg and THPP, pyrogallol 1 to 10 mg/kg did not modify the hypogastric response, while TMB 7 mg/kg significantly depressed the response. The rise of the intravesical pressure due to maximal stimulation of the pelvic nerve was prevented by the intravenous dose of 10 mg/kg of THB, THPP and pyrogallol in the increasing order.

The injection of acetylcholine 0.1 µg/kg into the iliac artery of anesthetized dog produced a transient rise of the intravesical pressure. The acetylcholine response was significantly depressed by THB 50 mg/kg or TMB 10 mg/kg. More than 1 hour was required
before a complete recovery of acetylcholine response was attained (Fig. 2).

b. Ureter: The rhythmic contraction of the isolated guinea-pig ureter was increased in amplitude and frequency after application of adrenaline and noradrenaline in concentrations above $5 \times 10^{-7}$. Pyrogallol, THB, 1,2,4-THB, TMB and THPP potentiated the action of catecholamine on the ureter. Pyrogallol was ten times more effective than THB. The results with the isolated rabbit and dog ureter were essentially similar. In addition, the increased contraction of rabbit ureter treated with BaCl$_2$ $10^{-4}$ was significantly depressed by TMB $5 \times 10^{-5}$ but not by THB $10^{-4}$.

The perfusion flow of saline through the ureter of anesthetized dog was not affected by the intravenous injection of THB, THPP and TMB in doses below 10 mg/kg. However, the decrease of flow rate due to the injection of BaCl$_2$ 10 mg/kg was prevented by THB 1 mg/kg.

The intraureteral pressure of anesthetized dog showed regular, rhythmic changes.

Fig. 2. The effect of 1,3,5-trihydroxybenzene (THB) and 1,3,5-trimethoxybenzene (TMB) on the contraction of urinary bladder in response to intraarterial injection of acetylcholine (ACh) in anesthetized dog.

Fig. 3. The effect of catecholamines on the intraureteral pressure of anesthetized dog.
The intravenous injection of adrenaline or noradrenaline 5 μg/kg lowered the pressure and decreased the rhythmic undulation. Isoproterenol was less effective than adrenaline or noradrenaline in the inhibitory action on the ureteral tone and movements (Fig. 3). The injection of tolazoline 5 mg/kg blocked the inhibitory action of catecholamines (Fig. 4). In contrast, propranolol 2 mg/kg did not affect the action of catecholamines. The injection of THPP, THB and pyrogallol at the dose of 10 mg/kg lowered the pressure and decreased the rhythmic undulation as did catecholamines. Pyrogallol was most potent and THB was least. Prior injection of THPP or THB in doses of 1, 5 and 10 mg/kg resulted in dose-dependent potentiation of catecholamine action.

3. Effects on gallbladder and bile duct

The intravesical pressure of the isolated dog gallbladder was not affected by pyrogallol 10^{-5}, THB 10^{-4} or TMB 2 \times 10^{-9}. But these agents potentiated a transient fall of the pressure due to adrenaline 10^{-6}. The sustained rise of the pressure due to acetylcholine 5 \times 10^{-7} was antagonized by adrenaline 10^{-6} or TMB 10^{-4} but not by THB 10^{-4}. The isolated guinea-pig gallbladder responded with a slight relaxation to adrenaline or noradrenaline in concentrations above 10^{-9}. THPP, THB and pyrogallol did not affect the tone of gallbladder, but slightly potentiated the catecholamine-induced relaxation. The guinea-pig gallbladder responded to acetylcholine in a concentration as low as 5 \times 10^{-9} with a sustained contraction. The contractile response to acetylcholine was partially to completely blocked by pretreatment with THB 10^{-4}, THPP 10^{-4}, pyrogallol or TMB 10^{-4}.

The intravenous injection of 1 to 10 μg/kg of adrenaline or noradrenaline to anesthetized dog mostly increased a perfusion flow through the bile duct, but in some cases decreased it. The prior injection of tolazoline 5 mg/kg potentiated the flow increase due to catecholamines. When the amines decreased the flow, tolazoline reversed the
response to an increase. The increase in flow rate by catecholamines was not influenced by prior injection of propranolol 2 mg/kg. The intravenous injection of 1 to 10 mg/kg of THPP or THB produced an increase in bile flow (Fig. 5) and potentiation of the action of catecholamines.

4. Effects on uterus and vas deferens

Pyrogallol in concentrations above $10^{-7}$ and TMB above $5 \times 10^{-4}$ decreased the rhythmic contraction of the isolated rat or guinea-pig uterus, while THB and THPP even in a concentration of $10^{-4}$ was without effect. The transient depression of rhythmic contraction of the rat uterus due to adrenaline $10^{-8}$ and $10^{-9}$ or noradrenaline $10^{-7}$ and $5 \times 10^{-8}$ was significantly potentiated by pretreatment with pyrogallol, THPP or THB.
The contractile response of the isolated rat uterus in De Jalon solution to acetylcholine $10^{-7}$ was significantly depressed by pyrogallol $10^{-6}$, THPP $10^{-5}$ or THB $2 \times 10^{-4}$.

The rhythmic contraction of the isolated vas deferens of rat was not affected by pyrogallol $10^{-6}$ or THB $10^{-4}$. However, pretreatment with either agent $10^{-6}$ potentiated the stimulating action of adrenaline $5 \times 10^{-7}$ and noradrenaline $5 \times 10^{-6}$. Pyrogallol was more effective in this respect.

5. Effects on intestinal tract

The isolated rabbit intestine responded to THB and TMB $10^{-4}$ with a relaxation of tone and a decrease in amplitude of contraction. However, both compounds were without effect on the inhibitory action of adrenaline $10^{-7}$.

The isolated rat intestine also responded to THB, TMB, THPP and pyrogallol above $10^{-6}$ with a relaxation and a decrease in amplitude of contraction. These agents potentiated to a various degree the inhibitory action of adrenaline.

DISCUSSION

Although there exists double innervation to the urinary tract, bile ducts and pelvic organs, the physiological significance of the role of the sympathetic and parasympathetic nerves on the motor activity of these organs is still unsettled. The response of the urinary bladder to stimulation of the pelvic nerve is atropine-resistant (9, 10). Stimulation of the parasympathetic nerve produces contraction of the gallbladder and relaxation of the sphincter of Oddi, while the sympathetic nerve inhibits the rhythmic contraction of the bladder (12). On the other hand, motility and tone of the gallbladder and Oddi sphincter were reported to be regulated more strongly by cholecystokinin released by acidic stimulation of the duodenum (13). However, no reliable drug has appeared to attenuate the spastic disorders of these organs (14).

Clinical effectiveness of THB and THPP against spastic disorders of the urinary tract, bile ducts and uterus has been widely recognized. Based on the findings that the COMT inhibitory action of these compounds parallels with their spasmylytic activity, Hattori et al. (8) have concluded that the spasmolysis derives from adrenergic mechanism with a possible accumulation of sympathetic neurotransmitter in tissues. The present report deals with the pharmacodynamic actions of THB and THPP, mainly, on the urinary tract, bile ducts and pelvic organs.

In anesthetized dogs and rabbits, THPP as well as pyrogallol produced a slight and transient rise of blood pressure only after large doses, but THB did not affect the blood pressure level. However, pretreatment with either agent potentiated the pressor response to exogenously administered catecholamines. The potentiation was most marked after pyrogallol and least after THB. The order of potentiating action of trihydroxybenzene derivatives roughly parallels with that of COMT inhibitory activities in vitro (7). The trihydroxybenzenes produced weak positive inotropic and chronotropic effects in isolated rabbit atria. The potentiation of action of catecholamines was also confirmed. Similar
potentiating action was seen in isolated rat and guinea-pig urinary bladder, isolated guinea-pig ureter, isolated dog and guinea-pig gallbladder, isolated rat uterus, vas deferens and intestine. The potentiation of excitatory or inhibitory action of catecholamines on smooth muscle organs was in a close correlation to COMT inhibitory activities of trihydroxybenzenes. In addition, these compounds by themselves showed sympathomimetic actions in these smooth muscle organs after application of high concentrations.

Since the rise of intravesical pressure of the urinary bladder in response to hypogastric nerve stimulation was not affected by hexamethonium, the nerve stimulated would be postganglionic in nature. Pyrogallol, THPP and THB did not affect the hypogastric response. The reason of lack of modification of hypogastric response was not elucidated in the present experiments. On the other hand, these trihydroxybenzenes depressed the rise of intravesical pressure due to pelvic nerve stimulation or injected acetylcholine. These facts suggest that the adrenergic relaxant effect of trihydroxybenzenes, probably, on the sphincter muscle of the urinary bladder does not occur unless the cholinergically innervated detrusor muscles contract for the elevation of intravesical pressure.

The perfusion flow in the ureter of anesthetized dog was not affected by trihydroxybenzenes, but the BaCl₂-induced decrease of flow was prevented. The intravenously administered catecholamines lowered the intrareteral pressure and decreased the rhythmic contractions. Since the inhibitory effect of isoproterenol was less than that of adrenaline or noradrenaline, and these effects were blocked by tolazoline, the inhibitory actions should be due to stimulation of the adrenergic α receptor. Pyrogallol, THPP and THB mimicked catecholamine in producing the inhibition of ureteral motility and tone, and potentiated the action of catecholamines.

Injection of THPP as well as THB increased the perfusion flow through the bile duct in anesthetized dogs. Catecholamines produced variable effects on the flow. Prior injection of tolazoline reversed the decrease of flow and potentiated the increase due to catecholamines. Therefore, it is possible that contraction of the sphincter muscle of Oddi with a subsequent decrease of bile flow after catecholamines is derived from adrenergic α receptor mechanism. On the other hand, the nature of relaxation of the sphincter muscle with a subsequent increase of flow remains to be settled in the present experiments. However, at any rate the action of catecholamines on the in situ dog bile duct was potentiated by trihydroxybenzenes.

SUMMARY

The pharmacodynamic actions of 1,3,5-trihydroxybenzene (THB) and 2,4,6-trihydroxy-1-propiophenone (THPP) on the smooth muscle organs were tested in an attempt to know whether spasmolytic actions of trihydroxybenzenes are correlated with COMT inhibition and subsequent potentiation of the action of catecholamines.

In anesthetized dogs and rabbits, THB, THPP and pyrogallol potentiated the pressor response to exogenously administered catecholamines. These compounds exerted weak positive inotropic and chronotropic effects on isolated rabbit atria, and potentiated the
action of catecholamines. The inhibitory actions of catecholamines in isolated rat and guinea-pig urinary bladder, isolated dog and guinea-pig gallbladder, isolated rat uterus and intestine were potentiated by the trihydroxybenzenes. The excitatory action in isolated guinea-pig ureter and isolated rat vas deferens was also potentiated. Pyrogallol, THPP and THB did not affect the contraction of urinary bladder in response to hypogastric nerve stimulation of anesthetized dog, while these compounds depressed the response to pelvic nerve stimulation or injected acetylcholine. The three compounds mimicked catecholamines in producing the inhibition of ureteral motility and tone of anesthetized dog, and potentiated the action of catecholamines. Injection of THPP and THB increased the perfusion flow through the bile duct in anesthetized dogs and potentiated the flow increase caused by catecholamines. The order of potency of pharmacodynamic actions described parallels with that of COMT inhibition in vitro.

REFERENCES

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