ADRENERGIC MECHANISM OF THE DRUGS. SYSTEMIC ARTERIAL PRESSURE CHANGES CAUSED BY INTRAVENOUS INJECTION OF RESERPINE FOLLOWING THE ADMINISTRATION OF PHENIPRAZINE AND PYROGALLOL IN ANESTHETIZED DOGS

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It has been reported that reserpine causes a prolonged hypertension after the pretreatment of ephedrine and metamphetamine. This hypertension was observed in the previous experiments and the mechanism of the drugs producing the hypertension was discussed.

Pheniprazine was proposed as a monoamine oxidase inhibitor by Horita, and reserpine produced a prolonged hypertension after the administration of pheniprazine. A similar pheniprazine-induced reserpine hypertension was recognized in the present experiments, however, this hypertension was clearly different from the reserpine hypertension after ephedrine and metamphetamine.

Furthermore, pyrogallol (a catechol-O-methyltransferase inhibitor) was used prior to the administration of reserpine.

Additionally, the influence of bilateral adrenalectomy and the pheniprazine tachyphylactic dog were tested, and the mechanism of the drugs, especially the catecholamine releasing action of the drugs were discussed in this paper.

METHODS

Experimental animals were 38 mongrel dogs of either sex weighing 6 to 11.5 kg anesthetized with morphine 10 mg/kg intramuscularly and pentobarbital sodium 15 mg/kg intravenously. Atropine 1 mg/kg was administered intramuscularly with morphine. Carotid arterial pressure was recorded on a smoked paper kymograph using a mercury manometer.

Reserpine (Triserpin, Torii Seiyaku Co.) (0.1%), pheniprazine (Catron, Chugai Seiyaku Co.) (1%), pyrogallol (Yoneyama Chemical Industries Ltd.) (20%), cocaine (Cocaine hydrochloride, Sankyo Seiyaku Co.) (0.5%), tolazoline (2-benzyl imidazole

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hydrochloride, Yamanouchi Seiyaku Co. (2%) were used in this experiments. All the drugs were diluted in physiological saline solution except reserpine, and the injections were made into the femoral vein.

The other experimental procedures were the same as in the previous report\textsuperscript{22}.

RESULTS

1) Systemic arterial pressure changes produced by the acute reserpine treatment following the administration of pheniprazine and pyrogallol.

The intravenous injection of reserpine 1 mg/kg produced a prolonged hypertension with an initial rapid fall of pressure. This dose of reserpine was used throughout the experiments.

\textit{Pheniprazine}

Pheniprazine 0.01 mg/kg caused no change of blood pressure and reserpine 1 mg/kg produced a very slight and gradual increase of pressure ranging from 6 to 12 mmHg. The intravenous administration of pheniprazine 0.1–1 mg/kg produced a pressor responses ranging from 6 to 118 mmHg which are smaller increases than those with the same dose of ephedrine and metamphetamine. The reserpine hypertension (5–109 mmHg) occurred after the premedication of doses between 0.1–1 mg/kg of pheniprazine. However, this hypertension was much slower and of a less magnitude than that following the ephedrine or metamphetamine treatments in the previous report\textsuperscript{22}. Sometimes, it continued to stay a little higher than the original level over a period of 10 min (Fig. 1A). In 3 cases using doses of pheniprazine over 0.1 mg/kg, reserpine caused only a prolonged blood pressure lowering, the same as the results from the injection of reserpine alone. This reserpine hypertension was diminished to less than a half degree after 5 hrs of pheniprazine 1 mg/kg compared with the response to the immediate administration of reserpine after pheniprazine 1 mg/kg.

\textit{Pyrogallol}

After a large dose of pyrogallol 50 mg/kg, the blood pressure fell 8–28 mmHg under control level. The administration of reserpine after pyrogallol produced only a slight pressor response (17–24 mmHg) (Fig. 1B). This was a much slower, gradual increase than that of the average pheniprazine-induced reserpine hypertension, and it still increased continuously even after 20 min. However, in 1 out of 3 experiments, reserpine produced only a hypotension after pyrogallol. Reserpine produced no hypertension after 5 hrs of the drug.

2) Tachyphylactic response of reserpine.

After recovery of the above-mentioned reserpine hypertension, reserpine was injected repeatedly. The hypertension was not observed with the second does of reserpine.

3) The influence of bilateral adrenalectomy on pheniprazine and pyrogallol-induced reserpine hypertension.

On the average reserpine hypertension after pheniprazine 0.5 mg/kg was not significantly affected by bilateral adrenalectomy however, in 1 out of 3, it was augmented in comparison with the effect of normal dogs (Fig. 2).
Fig. 1. Systemic arterial pressure changes caused by pheniprazine or pyrogallol and reserpine.
A. Pheniprazine (0.5 mg/kg)-induced reserpine (1 mg/kg) hypertension.
B. Pyrogallol (50 mg/kg)-induced reserpine (1 mg/kg) hypertension. Scale is in mmHg. Time interval is 10 seconds. Drugs were injected at the arrow intravenously. Remarks are the same as those of Fig. 2, 3 and 4.

Fig. 2. Adrenalectomized dog. Pheniprazine (0.5 mg/kg)-induced reserpine (1 mg/kg) hypertension.
Pyrogallol–induced reserpine hypertension was not clearly observed after bilateral adrenalectomy.

4) The effect of tolazoline and cocaine on the reserpine hypertension after pheniprazine and pyrogallol.

A. The effect of tolazoline.

The doses of 0.5–1 mg/kg of pheniprazine and 50 mg/kg of pyrogallol were administered prior to reserpine, and tolazoline 10 mg/kg was injected at the height of the hypertension. The reserpine hypertension produced by pheniprazine and pyrogallol were abolished immediately after the administration of tolazoline at the height of the elevation and the blood pressure returned suddenly to the original level and even fell to lower levels.

B. The effect of cocaine.

Pheniprazine–induced reserpine hypertension was blocked by the administration of cocaine 5 mg/kg at the height of this elevation.

Reserpine hypertension after pyrogallol 50 mg/kg was not affected by cocaine, and it produced an additional increase of pressure just after the injection of cocaine (Fig.3). This response by cocaine producing the hypertension was quite differ from the results with ephedrine, metamphetamine and pheniprazine.

![Fig. 3. The effects of cocaine on the pyrogallol-induced reserpine hypertension.](image)

After the observation of the effect of pheniprazine 0.5 mg/kg, cocaine 5 mg/kg was injected. And then reserpine was administered 5 min after cocaine. Reserpine hypertension after pheniprazine was markedly reduced (15 mmHg in average within 10 min) and it was a very slow and slight increase as compared to that without cocaine.

Reserpine could not produce any hypertension after pyrogallol and cocaine. Cocaine after pyrogallol caused a marked and prolonged hypertension.

Cocaine 5 mg/kg caused a diphasic (slight pressor and depressor) blood pressure change in most cases. Pheniprazine 0.5 mg/kg after cocaine elicited only a small magnitude of pressor response and then reserpine still produced a hypertension but it was markedly reduced in comparison with the effect without cocaine and sometimes it was not clearly demonstrated (5 mmHg in average).
Reserpine produced only a slight blood pressure increase (10 mmHg in average within 10 min) after cocaine and pyrogallol. In these experiments, pyrogallol produced diphasic blood pressure changes (a initial fall about 15 mmHg and later hypertension about 15 mmHg) after cocaine.

5) Systemic arterial pressure changes produced by the acute reserpine treatment after pheniprazine tachyphylaxis.

Tachyphylaxis (blood pressure reversal) was produced when pheniprazine was injected repeatedly with the total doses of 6–11.7 mg/kg.

The reserpine hypertension (76 mmHg in average) could be seen in all animals.
after pheniprazine tachyphylaxis, and there was a much greater and faster increase than the response observed after the single dose of pheniprazine 0.5-1 mg/kg (Fig. 4). This was clearly different from the results obtained in the experiments with ephedrine or metamphetamine tachyphylaxis.

Fig. 5 represents an average time course change of pheniprazine and pyrogallol-induced reserpine hypertension, and also the blood pressure changes caused by reserpine after ephedrine, metamphetamine, tyramine and catecholamines.

Fig. 6 is a summarization of the blood pressure changes caused by reserpine after pheniprazine and pyrogallol in percentage.

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*Fig. 6.* The percentage changes of blood pressure caused by reserpine alone and reserpine after pheniprazine and pyrogallol with the different doses and several procedures. Vertical bar represents a standard error on 3 to 10 experiments.

R : Reserpine 1 mg/kg alone.

Ph : Reserpine after pheniprazine. From left to right.

- Reserpine after pheniprazine 0.01 mg/kg.
- \(\rightarrow\) 0.1 mg/kg.
- \(\rightarrow\) 0.5 mg/kg
- \(\rightarrow\) 1 mg/kg.
- \(\rightarrow\) 0.5 mg/kg. in adrenalectomized dogs.
- \(\rightarrow\) tachyphylaxis.
- \(\rightarrow\) 5 hrs after pheniprazine 0.5 mg/kg.
- \(\rightarrow\) after cocaine 5 mg/kg+pheniprazine 0.5 mg/kg and after pheniprazine 0.5 mg/kg+cocaine 5 mg/kg.

Py : Reserpine after pyrogallol. From left to right.

- Reserpine after pyrogallol 50 mg/kg.
- \(\rightarrow\) in adrenalectomized dogs.
- \(\rightarrow\) 5 hrs after pyrogallol 50 mg/kg.
- \(\rightarrow\) after cocaine 5 mg/kg+pyrogallol 50 mg/kg and after pyrogallol 50 mg/kg+cocaine 5 mg/kg.
DISCUSSION

In the previous paper it has been reported that reserpine produced only a prolonged blood pressure decrease when it was injected intravenously in anesthetized dogs as was also shown by others in early reports.

Furthermore, the mechanism of reserpine on the catecholamine storage sites was discussed, and it was presumed that the action of reserpine might be only a change of catecholamines storage energy to elicit an easy release of catecholamines from their storage sites. Thus catecholamines might be slowly released after reserpine by a normal stimulus of adrenergic nerve impulse from the center.

Pheniprazine and other monoamine oxidase inhibitors might have the releasing action of catecholamines from their storage sites.

In the present experiments the pheniprazine-induced reserpine hypertension increased much slower than that of the ephedrine- or metamphetamine-induced reserpine hypertension in the previous experiments. After tachyphylaxis of pheniprazine (reversal effect), reserpine caused a quicker and more prolonged hypertension in comparison with the hypertension after a single dose of pheniprazine. From these experimental results, it was shown that pheniprazine has a releasing action on catecholamines but its effect might less primarily than that of ephedrine or metamphetamine because the blood pressure changes caused by pheniprazine were very slight as compared with those of ephedrine or metamphetamine. However, after repeated doses of pheniprazine, a large volume of catecholamines could be released promptly by reserpine. Of course monoamine oxidase might be inhibited enough after pheniprazine tachyphylaxis and a large volume of indestructable catecholamines released by reserpine might produce a hypertension. Chessin et al. demonstrated that reserpine caused a prolonged hypertension after iproniazid (30 mg/kg, 3 divided doses in one day). This hypertension was blocked by chlorpromazine, and dibenamine was less effective than that of chlorpromazine. They concluded that the pressor response is due to the liberation of serotonin by reserpine.

In the present experiment, reserpine administration after pheniprazine showed the similar pressor effect as that with iproniazid, but it was completely blocked by tolazoline. This agrees with the hypothesis supported by Clementi et al. and Maxwell et al. that the pretreatment of monoamine oxidase inhibitors produced reserpine hypertension and it might be a rapid release of a sympathetic humoral factor by reserpine after the inhibitor. On the other hand, the potentiating effect of monoamine oxidase inhibitors on the responses of catecholamines is not due to the enzyme inhibition. It seems unlikely that the inhibition of monoamine oxidase play a role in the initial pressor response of pheniprazine. It is most likely that this hypertension caused by reserpine and the pretreatment of pheniprazine is due to the liberation of catecholamines, not serotonin, from the storage sites. Furthermore, pheniprazine accumulated in the catecholamines storage sites after the repeated doses of the drug (tachyphylaxis), and reserpine might releases catecholamines stronger than that after single dose of pheniprazine.

Pheniprazine or other monoamine oxidase inhibitors reduce the release of catecholamines caused by reserpine, while Spector et al. reported that monoamine oxidase inhibitors including pheniprazine have no blocking action on the release of catecholamines by reserpine.
On the other hand, norepinephrine content in the tissue was increased or the spontaneous release was blocked by monoamine oxidase inhibitors including pheniprazine, while Hertting et al. reported that pheniprazine has no effect on the tissue level of catecholamines. It might depend upon the doses of the drug and the time after the administration of drugs. The monoamine oxidase inhibitors markedly reduce the uptake of exogenous catecholamines from their storage sites. The effects of exogenously administered epinephrine and norepinephrine were potentiated or relatively prolonged by monoamine oxidase inhibitors. From the present experiments, reserpine hypertension after a single injection of pheniprazine was slow and gradual elevation of blood pressure.

The reduction of the catecholamines releasing action with monoamine oxidase inhibitors after repeated doses of pheniprazine, if it is considered, is probably reversed by reserpine, and the speed of the release with reserpine becomes much more rapid and faster than that after a single dose of pheniprazine and the concentrated catecholamines might act promptly on receptor sites.

When pheniprazine and reserpine were administered, catecholamines escaped to the outside of the storage sites, and they could not re-enter into the storage sites because the pheniprazine blocks the entry of the catecholamines. A relatively large volume of catecholamines act on receptor sites continuously, thus reserpine treatment after large doses of pheniprazine produces a marked and prolonged hypertension. This represents the idea that reserpine helps the release of catecholamines with pheniprazine as a “better releaser” and it produces the hypertension.

Pheniprazine caused a tachyphylaxis (reversal effect), and it suggests that pheniprazine accumulates inside the stores of catecholamines as briefly discussed in the previous paper using ephedrine and metamphetamine. However, it has some difficulty in binding to the stores because the development of tachyphylaxis with pheniprazine was more difficult than that with ephedrine or metamphetamine.

The pressor response of pheniprazine was markedly potentiated after reserpine, and it was a greater potentiation than that of ephedrine and metamphetamine as it will be presented in a later paper. It means that the catecholamine releasing action of pheniprazine might be potentiated markedly by reserpine because pheniprazine smoothly enters into the storage sites and releases catecholamines easily after reserpine.

Pheniprazine-induced reserpine hypertension was blocked by cocaine which was administered at the height of the blood pressure elevation. Cocaine reduced the hypertension when it was administered before reserpine. This might be due to the interference of the transport of the storage membrane with cocaine as a “membranedichtend” (membrane become thicker), and reserpine could prevent or reverse this cocaine effect as described in the previous paper.

The pretreatment of ganglionic blocking agents in the anesthetized dogs produced a prolonged hypertension with reserpine, therefore they concluded that this phenomenon is due to the release of sympathetic humor by reserpine because this hypertension was blocked by dibenzylzine.

The catecholamine content in the tissue is elevated after ganglionic blocking agents. The pressor responses of epinephrine and norepinephrine were potentiated after ganglionic blocking agents. Interruption of nerve impulses from the central region presumably interferes with the spontaneous release or depleting action of reserpine on norepinephrine stores. It is most likely that when the catecholamine
content was elevated after ganglionic blocking agents, a lesser volume of exogenous catecholamines could enter and bind in the storage because the stores were full of catecholamines. Thus the exogenous catecholamines in high amount act on the receptor sites promptly and cause a potentiating action. When the stores were full of catecholamines after the ganglionic blocking agents, reserpine suddenly relieved the binding energy of catecholamines and the concentrated catecholamines produced a hypertension by acting on the receptors. Although ganglionic blocking agents have a blocking effect on the catecholamine releasing action of reserpine, a prompt and rapid release of concentrated catecholamines from their storage sites might be a main reason for the production of reserpine hypertension after ganglionic blocking agents.

Furthermore, the released catecholamines could not re-enter into their storage sites while reserpine was acting on it. It also produced a hypertension because the released catecholamines all act on the receptor sites.

Monoamine oxidase inhibitors or ephedrine like substances have a ganglionic blocking action and this effect might be involved partly in the mechanism producing the reserpine hypertension that is similar to that after ganglionic blocking agents.

Since the demonstration of Axelrod et al. and La Brosse et al., it has been recognized that catechol-0-methyl-transferase (COMT) is an enzyme of destruction for free circulating catecholamines.

In the present experiments, only a slight blood pressure decrease was observed after pyrogallol (as a COMT inhibitor) and then reserpine produced a very slight and gradual increase of blood pressure. However, in some cases, only a hypotension similar to the effect of an injection of reserpine alone was observed. On the average the pressure change was not significantly different from the response with reserpine alone. This pyrogallol-induced reserpine hypertension was blocked by tolazoline, and it might show the involvement of catecholamines in producing this hypertension. It seems likely that reserpine releases catecholamines very slowly into the circulation from their storage sites. It might not be due to the release of catecholamines by pyrogallol because it has been shown that it has no releasing action on catecholamines although it elevates the concentration of catecholamines in these stores. Pyrogallol does not block the release of catecholamines with reserpine, and it not prevents the uptake of circulating catecholamines.

Cocaine could not block this pyrogallol-induced reserpine hypertension, rather cocaine produced a potentiated blood pressure rise after these drugs in the experiments. Also, the depressor effect of pyrogallol was reversed to a very slight rise after reserpine and cocaine.

Pyrogallol prolongs the actions of exogenous or endogenous norepinephrine. When COMT is inhibited by pyrogallol, circulating catecholamines become very difficult to destruct and a high amount of catecholamines remain in the circulation and act on receptors.

After reserpine and cocaine, the released catecholamines could not re-enter the storage sites because it probably blocks their entry. So when catecholamines are released after pyrogallol and reserpine, these undestructable catecholamines in the circulation act on the receptors continuously and this produces a pyrogallol-induced reserpine hypertension. Reserpine might produce only the alteration of a catecholamine binding energy for the storage granules and terminal cells or their membranes. Thus reserpine hypertension after pyrogallol might not be markedly observed, because
reserpine produced a very gradual and slow increase of the circulating catecholamines and it might not be sufficient to produce a marked pressure rise and even COMT was completely inhibited by pyrogallol.

**SUMMARY**

Systemic arterial pressure changes produced by the acute reserpine treatment following the administration of pheniprazine or pyrogallol were studied with morphine and pentobarbital anesthetized and atropinized dogs.

1) Reserpine produced a very slow and gradual increase of systemic arterial pressure after the administration of pheniprazine. This reserpine hypertension was blocked by tolazoline, and it was blocked or reduced by cocaine. Pheniprazine-induced reserpine hypertension was not clearly altered by bilateral adrenalectomy. After pheniprazine tachyphylaxis (reversal effect) with the repeated doses of pheniprazine, reserpine hypertension became a much quicker and higher increase than that after a single injection of pheniprazine. Reserpine hypertension was reduced to a one half degree when reserpine was injected 5 hrs after pheniprazine.

2) After pyrogallol, reserpine produced a very slight and a negligible increase in the blood pressure, and in one case only a hypotension just like that of the administration of reserpine alone was observed. This hypertension was blocked by tolazoline but not cocaine. If cocaine was administered prior to pyrogallol and reserpine, or between these drugs, reserpine hypertension was reduced or not clearly observed. After bilateral adrenalectomy and pyrogallol or 5 hrs after pyrogallol, reserpine did not produce any hypertension.

It is concluded that catecholamines inside their storage sites were released very slowly after reserpine and this might be due to the changes of the catecholamines storage substances or energy in the stores.

Pheniprazine primarily has a very slight but a relatively long releasing action on catecholamines from their storage sites and reserpine helps the release of catecholamines after pheniprazine.

Pyrogallol might have no releasing action on catecholamines from their storage sites.

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