DEPRESSOR MECHANISM OF SYNTHETIC ACTH (50022)

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Tanaka et al. (1) have discovered the potent adrenocorticotropic activity of (Gly'')-ACTH (1-18)-octadecapeptide amide (50022), one of the synthetic polypeptide synthesized by Otsuka et al. (2), as well as its lypolytic and MSH activities.

Since ACTH has several extra-adrenal effects such as induction of stretching and yawning syndrome (3), sexual excitement (4), inhibition of the extinction of conditioned avoidance behaviour (5-7), hypoglycemic effect (8), in addition to its lypolytic and MSH activities, extensive pharmacological studies have been carried out on 50022 (9). The only noteworthy pharmacological effect observed with 50022 was a depressor effect seen in cats and rabbits on intravenous injection.

This experiment was undertaken to investigate the depressor mechanism of 50022 in experimental animals and to further compare the vascular effect of 50022 with that of vasoactive polypeptides.

METHODS AND MATERIALS

Under urethane anesthesia (1 g/kg s.c. in rabbits, pentobarbital at 25 mg/kg i.p. with urethane in cats), a glass cannula was inserted into the common carotid artery or femoral artery to record blood pressure, using an electric manometer (MP-4T, Nihon Khoden Kogyo), and another cannula was inserted into the trachea to record the respiration curve using a "respiration pick-up" (N. K. K.). The II lead of the ECG was also recorded using an AC preamplifier (RB-2, N. K. K.). Each signal was registered on a multipurpose polygraph (RM-150, N. K. K.). For recording the femoral blood pressure in some rabbits, its spinal cord and both vagi were cut in the cervical region under the artificial respiration. In the spinal rabbits angiotensin II was constantly infused into the femoral vein with an infusion pump (KN-202, Natsume) in order to maintain the blood pressure at control level. Contractions of nictitating membrane caused by the electrical stimulation of the preganglionic nerve fiber of cervical sympathetics with a bipolar silver electrode and a rectangular stimulator (MSE-3, N. K. K.) were recorded on the polygraph using an F-D pick up (N. K. K.).

Cardiac effects of the compounds in rabbits were observed both in vitro (Magnus method) and in vivo (Nuki's cardiotambour method) (10) experiments. In this case, carotid blood pressure was recorded on a rotating kymograph paper with a mercuric
manometer.

Three kinds of isolated blood vessels of rabbit—ear, hind quarter, mesenterium—were perfused with the heparinized blood (5000 unit/rabbit) and the effects of compounds on the drop number of perfusate in a given time or on the perfusion pressure were compared with those of the standard agents. In the perfusion of isolated whole ear vessels and marginal ear vein (11), the arterial blood collected by Hirako's method (12) was perfused at a constant pressure at 25°C (Krawkow-Pissenski's method). Isolated car artery, jejunal vessels in the ileum region and vessels of a hind quarter were perfused with the diluted arterial blood by the constant perfusion pump at 37.5°C (CV-2, Tokyo Kagaku Seiki). In these experiments, arterial blood was sampled from a carotid artery before the isolation of respective organs. Arterial blood was diluted ten times with Tyrode solution after filtration through several sheets of gauze and vigorously oxygenated before use. More details on the experimental methods are given with the respective results.

**Materials:** (Gly')-ACTH (1-18)-octadecapeptide amide 6 acetate (50022), 50022-S; a 50022 preparation which contained 50022 0.4 mg, mannitol 10 mg and benzalkonium chloride 0.02 mg, ACTH (Organon), angiotensin II (Institude for Protein Research, Osaka University), bradykinin triacetate (same institute as angiotensin II), epinephrine chloride (Sankyo), acetylcholine chloride (Daichi Seiyaku), phenoxybenzamine hydrochloride (Tokyo Kasei), propranolol (Sumitomo Kagaku), atropine sulfate (Merck), histamine diphosphate (Wako Junyaku), promethazine hydrochloride (Shionogi Seiyaku), isoproterenol hydrochloride (Nikken Kagaku), papaverine hydrochloride (Wako Junyaku).

In the current studies, the biological potency of 50022 was conveniently expressed...
in the unit with such correlation that one mg of 50022 was equivalent to 100 unit of ACTH.

In vivo experiments, these materials dissolved in the physiological saline solution were intravenously administered through a polyethylene tube into the femoral vein. In the perfusion experiment, however, drug solution in a volume of 0.05 or 0.1 ml was applied through an elastic perfusion tube just above the perfusion cannula.

RESULTS

1. Depressor effect of 50022 and ACTH

*In rabbits:* Depressor effect of 50022 was observed just after the intravenous administration and its maximal effect was about one minute after the injection (Fig. 1). Minimal effective dose of 50022 was 0.4 unit/kg in the intravenous administration and its depressor effect was dose-dependent. One of the characteristics of depressor effect of 50022 is that a more marked depressor effect was observed in the diastolic pressure than in the systolic pressure (Fig. 2). Also the recovery of diastolic pressure to the control

![Graphs showing depressor effects of 50022 and ACTH in rabbits.](image-url)

**Fig. 2.** Time course of the depressor effects of 50022 and ACTH in rabbits. (A): maximal fall of systolic pressure, (B): maximal fall of diastolic pressure.
level was very slow compared with that of a systolic one. Though an almost equivalent depressor effect was observed by the intravenous administration of ACTH, about five minutes were required to attain the maximal depressor effect. Slight increases of respiratory rate and heart rate accompanied the depressor effect of 50022 but no abnormal ECG II pattern was observed. Atropinization (2 mg/kg i.v.) caused no effect on the depressor action of 50022 or AGTH in two rabbits.

Depressor effect of 50022 in the spinal rabbit—Depressor effect of 50022 was also observed in the two vagotomized spinal rabbits under the continuous infusion of angiotensin II (1.7–4.2 μg/min) into the femoral vein (Fig. 3). Respiratory arrest caused by the spinal section at cervical region was prevented with the artificial respiration.

Effect on the contraction of rabbit nictitating membrane—A slight inhibition of the contraction of the nictitating membrane of rabbits was transiently noticed on the intravenous administration of 50022 at a dose of 2 or 8 unit/kg (Fig. 3). The inhibitory effect of hexamethonium on the contraction of nictitating membrane was more pronounced and lasted more than thirty minutes.

In cats: Dose dependent depressor effect was recognized with 50022-S by the intravenous administration (Table 1). On the other hand, the effect of 2–50 unit/kg of ACTH on blood pressure in cats was various—fall only, fall followed by rise, and rise only (9). As the additional agent of 50022-S—a mixture of benzalkonium chloride and mannitol—also had some depressor effect, the depressor effect of 50022 itself in cats
### Table 1. Effects of 50022-S on the respiration, carotid blood pressure and heart rate in cats.

| Compound | Dose (unit/kg) | No. of exp. | Respiration (rate/min) | Blood pressure (mmHg) | Heart rate (beats/min) |
|----------|---------------|-------------|------------------------|-----------------------|------------------------|
|          |               |             | Control | After | Dur. (min) | Control | Maximal change | Dur. (min) | Control | After | Dur. (min) |
| 2        | 5             | 25.2 ± 2.3  | 25.8 ± 2.3 | 1.0 (1) |           | 164.2 ± 4.3 | -16.5 ± 1.8→ 8.3 ± 0 (2) | 0.4→1.7 | 186.6 ± 6.4 | 166.6 ± 6.0 |           |
| 10       | 5             | 25.2 ± 2.4  | 26.2 ± 2.8 | 1.8 (2) |           | 177.6 ± 7.9 | -25.0 ± 0 →+13.4 ± 5.0 (2) | 0.4→3.5 | 195.0 ± 7.6 | 199.2 ± 8.3 | 1 (1) |
| 50       | 5             | 26.2 ± 2.8  | 32.6 ± 3.1 | 3.8     |           | 178.1 ± 4.8 | -36.7 ± 5.4 | 8.3       | 197.8 ± 7.7 | 200.8 ± 5.7 | 5.4   |
| 0.2 ml/kg| 2             | 27.0 ± 3.0  | 26.0 ± 0  | 4.5     |           | 178.6 ± 7.1 | -14.2 ± 2.2→+ 7.2 ± 1.1 | 0.4→0.9 | 204.0 ± 2.0 | 205.5 ± 2.5 | -     |
| Add. agent|              |             | 117.4 ± 9.2 | 183.0 ± 8.4 | -22.4 ± 4.1→+ 8.2 ± 1.2 (2) | 0.7→4.0 | 215.3 ± 6.0 | 214.0 ± 5.7 | 3 (2) |
| 1.0 ml/kg| 3             | 30.0 ± 7.0  | 38.0 ± 6.1 | 3.2     |           | 119.0 ± 6.0 | -36.8 ± 6.2→+ 8.2 ± 2.1 (2) | 0.5→3.5 | 36.7 | (1) | 0.6 |

1) : systolic pressure, 2) : diastolic pressure, (+) : rise, (-) : fall. 3) : standard error. 4) : saline solution of additional agent (mannitol, benzalkonium chloride) contained in 50022-S preparation in a dose of 10 or 50 unit/kg. Numerals in the parenthesis show the number of rabbits responded and side arrows indicate biphasic responses.
was very weak compared with that of 50022 in rabbits. The depressor effect of 50022-S in cats disappeared after the pretreatment of promethazine, though that in rabbits was not influenced by the same treatment (Fig. 4).

2. Effect on the cardiac movements

In vitro experiment: Effects of 50022-S and ACTH on the spontaneous movements of rabbit isolated right atrium were compared following Magnus’ method. Krebs-Ringerbicarbonate solution gassed with 95% O₂ and 5% CO₂ was used as a bathing fluid at 30°C. Neither inotropic nor chronotropic effects were observed in the rabbit isolated atrium by the administration of 50022-S or ACTH in the concentration of 0.5 unit/ml.

In vivo experiment: Depressor effect of 50022 (0.1 unit/kg i.v.) was followed by the
FIG. 5. Effect of 50022 on the rabbit cardiac movements in situ (Nuki's cardiotambour method).

Resp : respiratory movements. B.P.: carotid blood pressure. C. Side : movements of cardiac lateral wall, C. Apex : movements of cardiac apex.

| Compounds | Dose | Blood pressure (mmHg) | Cardiac movement | Dur. (min) | Side | Dur. (min) | Apex | Dur. (min) |
|-----------|------|-----------------------|------------------|------------|------|------------|------|------------|
|           |      | Control Maximal change |                 |            |      |            |      |            |
| 0.1 unit/kg |      | 67.0 -18.0            | 4.0              | ↓           | 3.5  |            |      |            |
| 3.2*       | 1.2* | 70.0 -27.0            | 9.3              | ↓(2)       | 7.3  |            |      |            |
| 50022 0.5 µ | 2.6  | 67.0 -34.7            | >30.0            | ↓(2)       | 13   | ↓(2)       | 6.5  |            |
| 2 µ        | 1.8  | 1.5                   |                  | ↓(1)       | 13   | ↓(2)       | 14.3 |            |
| ACh 1 µg/kg|      | 65.3 -30.7            | 2.0              | ↓           | 2.5  | ↓(1)       | 3.5  |            |
| Epi 2 µg/kg|      | 66.0 +22.7 -7.0       | 0.5→2.7          | ↑→↑(2)     | 0.8→2.3 | ↑(2) | 2.0  |            |

Number of rabbits : 3, * : standard error, (+) : rise, (−) : fall, ↑ : increase, ↓ : decrease, ↓(↑)<↑↑(↓). Numerals in the parenthesis show the number of rabbits responded and side arrows indicate biphasic responses.
slight decrease of contraction amplitude on the cardiac lateral wall only, while the marked depressor effect of 50022 in higher doses caused a decrease of contraction amplitude both on the cardiac lateral wall and cardiac apex (Fig. 5, Table 2). Inhibitory effect on the former, however, always preceded that on the latter.

3. Effect on the peripheral blood vessels

In the rabbit isolated ear vessels: The effect of 50022-S on the drop number of perfusate in rabbit isolated whole ear vessels perfused with heparinized blood was compared with those of ACTH and other standard drugs. In the four experiments in which the vaso-
dilator effect of ACh was observed, 50022-S in 0.5 or 5 units gave an increase in drop number of perfusate followed by a marked decrease (Fig. 6). Compared with the few minutes duration of the vasodilator effect of 50022-S, its vasoconstrictor effect was long lasting. Decreases in drop number of perfusate were observed at about three minutes after ACTH administration, lasting about ten minutes.

In the constant flow perfusion of rabbit ear artery (133.9–112.0 mmHg), 50022 in 0.05 or 0.1 units transiently depressed the perfusion pressure but in higher doses it was followed by a marked elevation. (Fig. 7, a). Although the elevation of perfusion pressure by 50022 was almost completely antagonized by phenoxybenzamine at $10^{-6}$ g/ml, the effect of angiotensin II was not influenced by the presence of an $\alpha$-blocker (Fig. 7, b).
Constant pressure perfusion of the marginal ear vein showed that 50022 and angiotensin II had the same vascular effects as observed in the perfusion of the ear artery, while bradykinin caused marked constriction of the ear vein and not in the artery as reported in the Hirako's paper (12) (Fig. 8). 50022 in a dose of 0.5 units caused a transient increase of drop number of perfusate followed by a decrease in three preparations.

In the vessels of a hind quarter and the jejunal vessels: In the constant flow perfusion of an isolated hind quarter (128.8–55.2 mmHg), 50022 in a dose of 0.5 or 5 units always depressed the perfusion pressure by about 10 mm Hg in four preparations.

![Fig. 9. Effect of 50022 on the perfusion pressure in the rabbit mesenteric vessels. Upper trace: venous outflow.](image)

**Table 3. Effects of 50022 and autonomic agents on the perfusion pressure in the rabbit mesenteric vessels.**

| No. of exp. | 50022 (↓) | ACh (↓) |
|-------------|-----------|---------|
|              | 0.05 unit | 0.5 unit | 5 unit | 1 μg | 10 μg |
| 1           | 24.7      | 21.7    | 12.1    | 36.2 | 30.0 |
| 2           | 10.6      | 15.1    | 14.6    | 25.4 | —    |
| 3           | 10.7      | 14.7    | 8.6     | 27.1 | —    |
| 4           | 20.0      | 29.3    | 25.9    | 21.7 | 30.5 |
| Average     | 16.5 ± 3.5 | 20.2 ± 3.4 | 15.3 ± 3.7 | 27.6 ± 3.1 | 30.3 |

| No. of exp. | Epi (↑) | Iso (↓) | Pap (↓) | P.P. (mmHg) |
|-------------|---------|---------|---------|-------------|
|              | 0.1 μg | 1 μg   | 10 μg  | 100 μg      |
| 1           | 10.3   | 44.8   | 10.6    | 38.5        | 146.0–91.3 |
| 2           | 19.7   | 19.0   | 11.7    | 22.9        | 117.3–78.2 |
| 3           | 8.3    | —      | 3.7     | 7.8         | 144.0–82.7 |
| 4           | 20.5   | 40.2   | 19.9    | 53.7        | 146.8–78.7 |
| Average     | 14.7 ± 3.1 | 34.7 ± 7.9 | 11.5 ± 3.3 | 30.7 ± 9.9 | 138.5–82.7 |

(↑), (↓): rise and fall of perfusion pressure (mmHg), *: standard error, Iso: isoproterenol, Pap: papaverine, P.P. shows the initial perfusion pressure.
these preparations, depressor effects of ACh (10 μg), isoproterenol (0.5 μg), papaverine (100 μg), bradykinin (0.1, 1.0 μg), and pressor effect of epinephrine (0.5 μg) were re-affirmed.

In the jejunal vessels as well as in the vessels of a hind quarter, moderate depression of the perfusion pressure was observed on the administration of 50022 (Fig. 9, Table 3). Even at a dose of 5 units, no elevation of perfusion pressure was observed in the perfusion of both hind quarter vessels and mesenteric vessels by the administration of 50022. No effect of 50022 on the venous outflow in the perfusion of mesenteric vessels was observed with the weight recorder (13).

![Graph showing repeated administration of 50022](image)

The effect of repeated administration of 50022 at ten minute intervals on the perfusion pressure in the mesenteric vessels and also the effect of propranolol pretreatment on the depressor effect of 50022 were studied following the scheme depicted in the Fig. 10. That is, heparinized arterial blood from the carotid artery pooled in the blood reservoir was perfused through a polyethylene tube into the jejunal artery with the constant flow pump. Almost no tachyphylaxis was observed in the depressor effect of 50022 in the perfusion of mesenteric vessels. After intra-arterial pretreatment with propranolol in a dose of 0.2 mg/kg which prevented the depressor effect of isoproterenol, depressor effect of 50022 was inhibited by about 70% in the three out of five experiments.

![Diagram showing experimental setup](image)
Maximal rise of blood corticoid level by the intravenous administration of 50022 was observed ten to twenty minutes after administration and the elevated corticoid level returned to control level in about sixty minutes in rats (1). The maximal depressor effect of 50022 was observed about one minute after intravenous administration and the depressor effect disappeared in ten to twenty minutes. The time course of the depressor effect of 50022 is quite different from that of corticoid release. So the depressor effect of 50022 is considered as one of the main extra-adrenal effects, except for its lypolytic and MSH activities.

Pretreatment with antihistaminics inhibited the depressor effect of 50022 in cats but not in rabbits. That is to say, species differences exist in the mechanism of depressor effect of 50022. Some histamine-like substances released in cats might contribute in the depressor effect of 50022 in cats. Depressor effect of 50022 in rabbits was least affected by atropinization and was observed in the vagotomized spinal rabbits. Inhibitory effect of 50022 on the sympathetic ganglion was considered negligible, because only a slight inhibition of contraction of nictitating membrane was transiently observed at one minute after the intravenous administration of 50022.

In the Nuki's cardiotambour method, movements of cardiac lateral wall and of the apex represent the blood volume in the cardiac cavity and the contractile force of the myocardium, respectively (10). On the intravenous administration of 50022 in smaller doses, only the movements of cardiac lateral wall were slightly inhibited, following depression of carotid blood pressure. With higher doses of 50022, however, the decrease of contraction amplitude of cardiac apex was also observed, following that of cardiac lateral wall. These results show that the inhibitory effect of 50022 on the cardiac movements was secondary to its depressor effect. Decrease of venous return due to peripheral vasodilatation might cause the decrease of blood volume in the cardiac cavity and thus inhibit the contraction of myocardium.

In the perfusion of rabbit isolated ear vessels with oxygenated Tyrode solution, only the vasoconstrictor effect of 50022 was recognized and the vasoconstriction disappeared on the pretreatment with phenoxybenzamine (9). In the heparinized blood perfusion of rabbit isolated ear vessels, vessels of an isolated hind quarter and mesenteric vessels, however, slight to moderate vasodilatation was always observed on the administration of 50022. Loss of the vascular tone in the perfusion experiment with Tyrode solution is considered to mask the vasodilator effect of 50022 in the preceding experiments.

According to Mellander and Johansson (14), blood distribution in the human body at rest shows that the visceral organs hold about 50% of total blood. So the depressor effect of 50022 is presumed to be due mainly to the peripheral vasodilatation of visceral organs.

Although only moderate vasodilatation was observed in the perfusion of both the hind quarter vessels and the mesenteric vessels, marked vasoconstriction preceeded by
the transient vasodilatation was noticed in the perfusion of rabbit ear on the administra-
tion of 50022. These results show that 50022 causes vasoconstriction via the \( \alpha \)-receptor in the \( \alpha \)-dominant blood vessels such as cutaneous vessels (15, 16), because the vasocon-
striction of the rabbit ear by 50022 was markedly inhibited by pretreatment with phenoxy-
benzamine. Further studies are needed to determine whether the vasoconstriction caused
by 50022 is due to 50022 itself or to any metabolite produced by plasma peptidase or
catecholamine release.

Vascular effects of 50022 are different from those of bradykinin in the following
two points: 1) vascular effects of 50022 were the same in both artery and vein, 2) almost
no tachyphylaxis was observed on the repeated administration to the mesenteric vessels.
It is noteworthy that propranolol pretreatment inhibited the vasodilator effect of 50022
in three out of five experiments. Selective \( \alpha \)-stimulant effect of 50022 on the blood ves-
sels may be concerned with the vasodilator effect of 50022.

Some ACTH metabolites are considered to be the cause of depressor effect of ACTH
in rabbits because about five minutes are required for its maximal depressor effect to
develop.

**SUMMARY**

The depressor effect of 50022 was observed in rabbits and cats, the effect in rabbit
being more pronounced than that in cats. Involvement of some histamine-like substances
are presumed in the depressor effect of 50022 in cats. The depressor effect in rabbit
by the administration of 50022 was mainly caused by the peripheral vasodilatation of
such blood vessels as visceral and striated muscle vessels, later followed by a cardiac in-
sufficiency. In the \( \alpha \)-dominant cutaneous vessels such as rabbit ear vessels, however,
marked vasoconstriction due to the excitation of \( \alpha \)-receptor, preceded by transient vaso-
dilatation, was observed on 50022 administration. Possible involvement of a selective
\( \beta \)-stimulant effect of 50022 on the blood vessels was suggested as a cause of the peripheral
vasodilator effect.

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