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16. Kazuo Watanabe: Some Pharmacological Factors involved in Formation and Prevention of Stress Ulcer in Rats.

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In the previous paper[1] the author described the method for the bioassay of antiulcerous effect using gastric ulcers caused by the stress in rats. The etiology of the stress ulcer is not yet established. On guessing from the complex etiology of human peptic ulcer, that of the stress ulcer in rats, too, may be rather complicated. For the studies on the pharmacology of antiulcerous drugs, it will be necessary to know the role of pharmacological factors affecting the ulcer formation as far as possible. In this paper the effect of stress on some gastric functions, mainly on gastric motility, secretion, blood circulation and defensive force of gastric mucosa against gastric juice was described. Then the importance of these factors was compared in relation to the pharmacology of antiulcerous drugs. The effects of some pharmacodynamic agents on these factors were also described.

Methods

1. Development of Stress Ulcer in Rats

Male rats weighing 150~180 g. were used. After fasting for 24 hr., rats were fixed on their backs by the limbs with thread on boards and immersed into water to the depth of the xiphoid. Temperature of water was kept at 25°. The stomachs of the rats were biopsied after 20 hours' stress and the severity of ulcer was recorded as −, + ∔, and ∔∔, which were described in the previous report.[1]

2. Vagotomy and Adrenalectomy

The vagus of the rats was sectioned at the level of the neck and the rats were inflicted the stress 24 hr. after the operation. Both adrenals were excised from the back of the rat, and soon after the operation the rats were inflicted the stress.

3. Gastric Motility under Stress

Pressure change in small rubber balloon fixed in the glandular stomach was recorded with water manometer. The balloon was inserted from the forestomach under light ether anesthesia. Initial pressure was kept at the height of 5 cm. H_2O. The rat attached with the balloon was kept in water bath of 38° for 2 hr. and when the effect of anesthesia and of the operation was diminished, the temperature of the bath was cooled to 25°. Under these conditions the body movement could be nearly eliminated from recording.

4. Estimation of Gastric Secretion

In the study on the effect of the stress, the procedure described by Shay[2] was employed. After fasting for 24 hr. the stomach of the rat was ligated at the pylorus under light ether anesthesia, and after the infliction of stress for 4 and 20 hr. the gastric juice in the stomach was collected. The volume,

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1) K. Takagi, Y. Kasuya, K. Watanabe: This Bulletin, **12**, 465 (1964).
2) H. Shay: Gastroenterology, **5**, 43 (1945).

total acid, free acid and pepsin activity of the gastric juice were estimated. Pepsin activity was assayed with the method of Anson.[3] In the other experiments, gastric acid secretion was recorded successively with the procedure described by Ghosh.[4] In this experiment *N*/4000 NaOH solution was perfused at the rate of about 1 ml./min. through the stomach from esophagus to pylorus of the rat which was anesthetized with urethane, and the pH change of the perfusate was recorded with the glass electrode pH meter. Drugs were injected into vena femoralis.

5. Measurement of the Blood Flow through Gastric Wall

The appropriate method to measure the blood flow through the small vessels is not yet established. Especially in the small animal as rats, the measurement of the gastric blood flow is difficult. A simple and primitive method was devised by the author. A fine polyethylene tube (about 0.1 mm. in diameter) was cannulated into one of the blanches of vena gastrica of the anesthetized and heparinized rat, and the other end of the tube was led to the small glass globe which was filled with saline. The time of the interval of dropping of the saline was measured. If the proper blanch of the vena gastrica was selected, the dropping interval would be about 60 sec. Under these conditions, blood loss was negligible and during experimental period of about 20 min. the interval was kept unchanged.

6. Acessment of Protective Ability of Gastric Mucosa

The rat was attached the cannula (3 mm. in diameter) in the forestomach and the pylorus was ligated. Through this cannula thin rubber tube was inserted and various perfusate was flowed into this tube. Perfusion velocity was kept at 1 ml./3 min. The operation was performed under ether anesthesia and 1 hr. after the operation the rats were inflicted the stress. After the stress for 20 hr. the severity of gastric ulcer was examined.

Results

1. Effect of Stress on Gastric Motility

Emotional and physical stress induced by immobilization and immersing in water gave the great influence on the motility of a stomach of a rat. As shown in Fig. 1

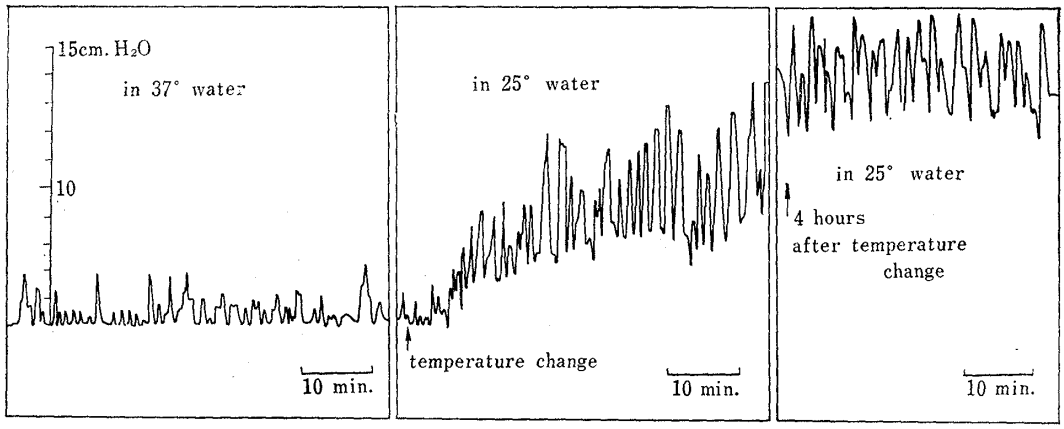


Fig. 1. Influence of Stress on Gastric Motility

Pressure change in the stomach was recorded with water manometer. Details of the method were described in the text.

pressure in the stomach rose soon after the temperature was lowered. The pressure in the abdominal cavity was recorded for reference in some experiments and no remarkable change was detected. These finding was assured with 5 animals. For the recording of gastric motility of conscious rats, movement of the body was obstructive, but 2 hours after the fixation, these movements were settled and it was possible to record almost only the contraction patterns of stomach.

3) M. L. Anson : J. Gen. Physiol., **22**, 79 (1939).
4) M. N. Ghosh, H. O. Schild : Brit. J. Pharmacol, **13**, 54 (1958).

2. Effect of Stress on Gastric Secretion

To examine the effect of stress on gastric secretion, pylorus ligated rats were employed. The stress was given to two groups of rats. One group was stressed for 4 hours and the other for 20 hours. Equal number of control groups were kept free from the stress after the ligation of the pylorus. Each groups were consisted of 10 animals. Gastric content of each rat was analysed for its volume, acidity, pH and pepsin activity. The results of this experiment were shown in Fig. 2. Four hours' stress caused hyperacidic secretion, though it had little effect on pepsin activity of gastric juice. The volume of gastric juice was somewhat decreased. But the differeces between the control and the stressed group diminished after 20 hours' stress. Namely, higher acidity and decreased volume of gastric juice of the stressed groups were not observed in the group which had been stressed for longer duration.

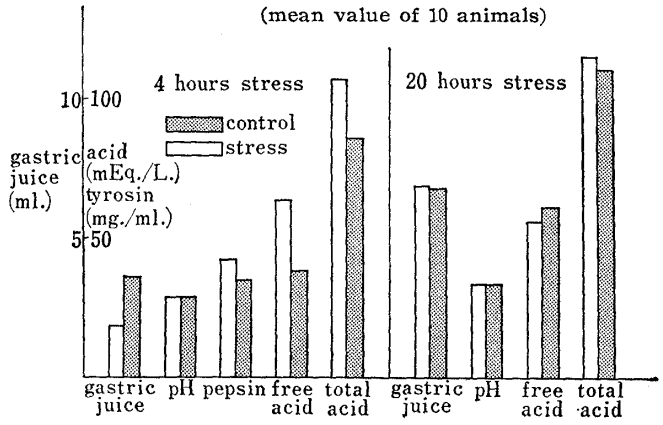


Fig. 2. Effect of Stress on Gastric Secretion

The stress was inflicted to pylorus ligated rats. Pepsin activity was represented as tyrosine equivalent according to the Anson's method.

3. Influence of Vagotomy and Adrenalectomy on Ulcer Formation by the Stress

Vagotomized or adrenalectomized rats were immobilized and immersed into water, and they were examined for ulcer formation after 20 hours. The result of this experiment was shown in Table I. The vagotomy resulted in complete depression of

Table I. Influence of Vagotomy and Adrenalectomy on Gastric Ulcer induced by Stress

Treatment	Number of animals	pH of gastric juice	Ulcer	
			incidence (%)	mean number
Vagotomy	10	4.2	0	0
Adrenalectomy	10	4.0	100	4.5
Sham operation	5	3.4	100	5.1

ulcer formation, while the adrenalectomy strenghened the severity of each ulcer. This result seemed to indicate the importance of parasympathetic nervous system in stress ulcer formation.

4. Effect of Some Pharmacodynamic Agents on Stress Ulcer Formation

Effects of histamine, physostigmine, methacholine, serotonin, adrenaline, noradrenaline, vasopressin, and dibenamine on stress ulcer formation were tested. These drugs were dissolved or suspended in 30% polyvinylpyrrolidone solution in expectation of prolonged action. The doses were somewhat excessive, but they would be necessary to prolong the action of drugs whose effects were transient. The drugs were injected subcutaneously 5 minutes before the stress. Each treatment was given to 10 animals. The control group was given the solvent in the same route. The result was shown in Table II. The histamine, physostigmine and methacholine groups

Table II. Effect of Some Pharmacodynamic Agents on Gastric Ulcer Induced by Stress

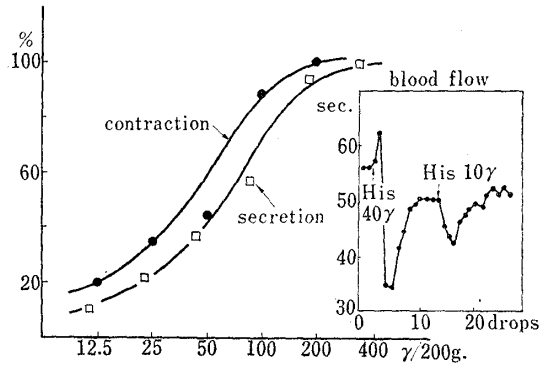
Drug	Dose mg./kg., s.c.	Ulcer severity ###	##	+	−	incidence	mean number of ulcers
Control	—	6	3	1	0	10/10	6.1
Histamine	200	9	1	0	0	10/10	9.1
Physostigmine	1	7	1	2	0	10/10	8.4
Methacholine	1	8	1	1	0	10/10	7.2
Serotonin	10	4	4	1	1	9/10	6.0
Adrenaline	5	4	3	3	0	10/10	4.0
Noradrenaline	5	5	2	2	1	9/10	4.9
Vasopressin	5 unit	5	4	0	1	9/10	6.6
Dibenamine	100	7	3	0	0	10/10	5.5

showed clearly severer ulcer formation than the control group. The vasoconstricting agents such as adrenaline, vasopressin and serotonin had little effect.

5. Effects of Histamine, Methacholine, Serotonin, and Vagus Stimulation on Gastric Functions

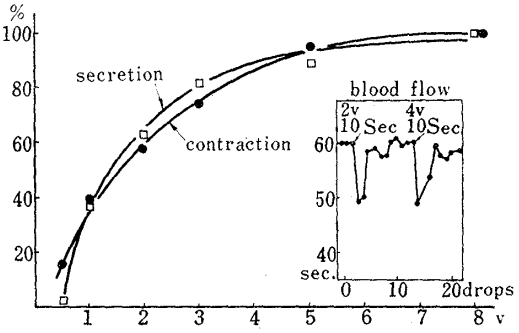
These experiments were performed to confirm the action of ulcerogenic drugs and the vagus on gastric functions. The action of these drugs on gastric secretion was tested with the Ghosh's method. The vagus was exposed at the neck and stimulated by the electrical stimulator.

a) Effects of histamine on gastric functions



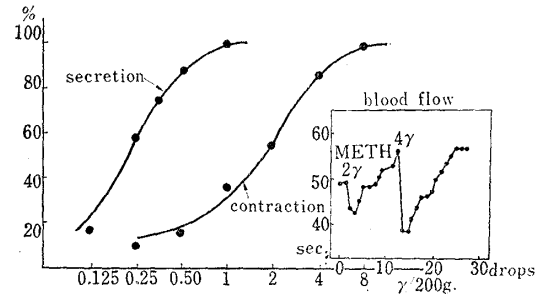
100% response is defined as the response induced by the highest dose of each treatment in the figure. The blood flow is represented as the interval (sec.) of each successive two drops.

b) Effects of electrical stimulation of vagus on gastric functions



electrical stimulation: duration; 5 msec.
frequency; 20 cycle/sec.

c) Effects of methacholine on gastric functions



d) Effects of serotonin on gastric functions

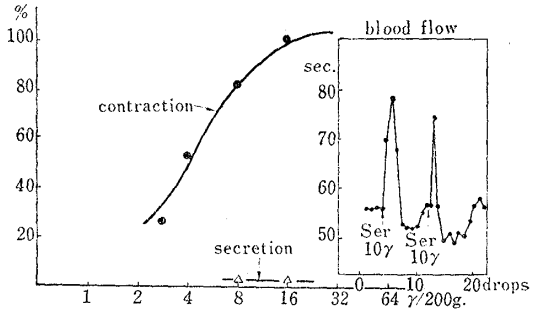


Fig. 3. Effects of Histamine, Methacholine, Serotonin, and Electrical Stimulation of Vagus on Gastric Functions

The results of this experiment were illustrated in Fig. 3 (a, b, c, and d). Histamine, methacholine and vagus stimulation showed considerable elevation of gastric motility and secretion, and both the effects were parallel. Blood circulation was increased by the three treatments.

6. Effects of Anticholinergic Drugs on Stress Ulcer and Relationships between Antiulcerous Effects and Spasmolytic or Antisecretory Action

Quantitative study on the relationship between antiulcerous effects and spasmolytic or antisecretory activity of a few anticholinergics was performed *in situ* in the rat. The results of this experiment were illustrated in Fig. 4, 5, and 6. Both effects equally related to the antiulcerous effects.

7. Effect of Stress on the Ability of Gastric Wall to defense the Aggressive Action of Gastric Juice

It is natural that aggressive effect of gastric juice is thought to be an important

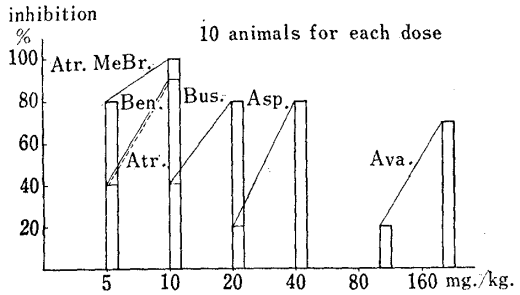


Fig. 4. Effect of Some Anticholinergics on Stress Ulcer in Rats

Ordinate: inhibition of incidence of stress ulcer (% of ulcer inhibited animals in a group of 10 animals)
Atr. MeBr.: atropine methylbromide
Atr.: atropine sulfate
Ben.: benactyzine
Bus.: hyoscyamine N-butyl bromide
Asp.: aspaminol
Ava.: avacan

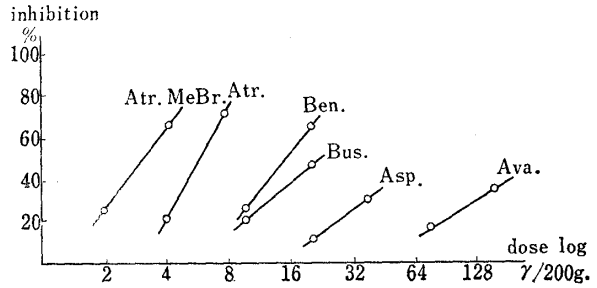


Fig. 5. Effects of Some Anticholinergics on Gastric Motility in Rats

Abbreviations of the drugs are same as those in Fig. 4.
Ordinate: percentage of inhibition against the contraction of stomach by the stimulation of methacholine (10 γ/kg., i.v.)
Details of the methods are described in the text.
Each point indicates the mean value of 5 experiments.

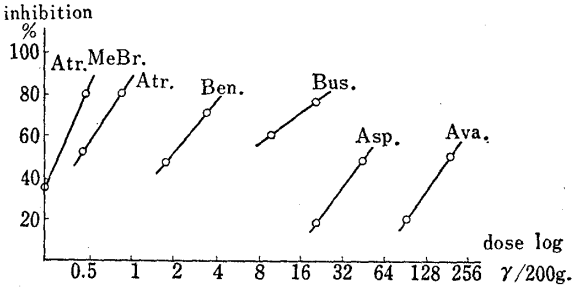


Fig. 6. Effects of Some Anticholinergics on Gastric Secretion in Rats

Abbreviation of the drugs are same as those in Fig. 4.
Ordinate: percentage of inhibition against gastric secretion caused by methacholine (2.5 γ/kg., i.v.)
Details of the methods are described in the text.
Each point indicates the mean value of 5 experiments.

factor for the gastric ulcer formation by stress. But much attention should be paid for defensive ability of gastric mucosa. From such a point of view, the author conceived the method to measure the defensive power against gastric juice. Principle of this method is to keep the aggressive action constant by perfusing gastric wall of a conscious rat with appropriate perfusate. As the perfusates, $N/10$ hydrochloric acid, $N/10$ sodium bicarbonate, and artificial gastric juice, which contains $N/10$ hydrochloric acid and 1% crude pepsin, were used. The results of this experiment were shown in Table Ⅲ. The animals which were exposed to the stress for 20 hours had suffered much severer ulcer than the rats which were anesthetized with urethane (1.5 g./kg., s.c.) and perfused with artificial gastric juice, but not exposed to the stress. Severity of ulceration of the two groups which were treated with $N/10$ hydrochloric acid or with the artificial gastric juice was approximately equal. This result indicates that

Table III. Effect of Stress and Various Perfusates on Ulcer Incidence of Rats

Treatment and perfusate	Ulcer				Incidence	Haemorrhage
	##	#	+	−		
Stress pepsin+*N*/10 HCl	6	0	0	0	6/6	+++
Stress *N*/10 HCl	6	0	0	0	6/6	+++
Stress pepsin+*N*/10 HCl atropine 10 mg./kg.	0	2	2	2	4/6	+
Urethane 1.5 g./kg. pepsin+*N*/10 HCl	0	2	1	3	3/6	+
Stress *N*/10 $NaHCO_3$	0	0	3	3	3/6	+

aggressive effect of acid is more important than the proteolytic activity of pepsin. Atropine (10 mg./kg., s.c.) remarkably inhibited the ulcer formation in the animals which were perfused with *N*/10 hydrochloric acid.

Discussion and Conclusion

Today antiulcerous effect of anticholinergics and other drugs can be evaluated quantitatively on some experimental peptic ulcer.[5,6] But it is not yet studied thoroughly to find which effects of antiulcerous drugs are of the primary importance. So the author intented to study the pharmacological factors involved in the etiology of experimental stress ulcer. The effects of ulcerogenic and antiulcerous drugs on these factors were also studied. First of all, effect of stress on gastric motility and secretion was examined. The stress promoted both of the two functions. With regard to gastric secretion, these results agreed with that of Brodie's[7] who had employed the rat with chronic fistula. Gastric motility was remarkably stimulated by the stress. These results suggest that the stress influences on gastric functions more strongly through the vagal path rather than the pituitary-adrenal system. This view was supported by the experiment in which the ulcer formation in the vagotomized and adrenalectomized rats was compared. In the vagotomized rats ulcer formation was completely inhibited, while in the adrenalectomized rats it became rather severe. Some authors[8] discussed the role of adrenal cortex in the etiology of the stress ulcer, but the clear explanation is not yet obtained.

The importance of gastric acid secretion for the formation of stress ulcer was confirmed by the experiment comparing the effects of pharmacodynamic agents on stress ulcer. Secretagogues like histamine, methacholine and physostigmine exerted the aggravating action on ulceration, and smooth muscle contracting or vasocontractile agents as serotonin, vasopressin and adrenaline did not show any remarkable effect. To avoid the criticism that the drug action was too transient to exhibit any effect, all drugs were given in excessive dose and in form of long durable preparation. And some of these drugs which aggravated stress ulcer formation were examined their action on gastric motility, secretion, and blood circulation. The result of this experiment gives another support for the above discussion where the role of gastric acid in formation of stress ulcer is emphasized.

5) C. Radouco-Thomas, D. Larue : Arzneim. Forsch., **10**, 588 (1960).
6) D. A. Brodie : Gastroenterology, **43**, 107 (1962).
7) D. A. Brodie, R. W. Marschall, O. M. Moreno : Am. J. Physiol., **202**, 812 (1962).
8) D. A. Brodie, R. W. Marschall, O. M. Moreno : Gastroenterology, **43**, 675 (1962).

The correlation between antiulcerous effect and other pharmacodynamic action of anticholinergics was studied quantitatively. Both of relaxing effect on gastric smooth muscle and depressive effect on gastric secretion were closely related to the antiulcerous activity of anticholinergics.

Though the evaluation of protective action of gastric mucosa against gastric juice has been considerd very important, the suitable method for the estimation of this action is not known. The author conceived a suitable method for this purpose. With this method one of the causative factor for the ulceration, namely the aggressive force of gastric juice, can be changed at will. In this experiment, it was made clear that stress causes the remarkable decrease of the defensive ability of gastric wall against gastric juice and that atropine seemed to have the effect inhibiting the decrease of it.

The author is greateful to Prof. K. Takagi and to Assist. Prof. Y. Kasuya for their guidance and encouragement.

Summary

The pharmacological factors which affect the stress ulcer formation were studied in rats. Some gastric functions, mainly gastric motility, secretion, blood circulation and protective action of gastric mucosa play the important roles in the ulcer formation induced by the stress which was inflicted by immobilization and immersing in water. Effect of some pharmacodynamic agents on the stress ulcer was examined, and it was found that the secretagogue action is more important than the other pharmacodynamic action tested. Effects of anticholinergics on the gastric functions were also studied, and the close relations between antiulcerous effect and depressive activity of gastric secretion and motility was confirmed. The protective action of gastric mucosa against gastric juice was estimated and the effect of stress and atropine on it was examined. Atropine seemed to have the effect inhibiting the decrease of the protective action of gastric mucosa.

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