Pharmacology Biochemistry & Behavior, Vol. 19, pp. 483–486, 1983. Ankho International Inc. Printed in the U.S.A.

Mucosal Damage Following Electrical Stimulation of the Anterior Cingulate Cortex and Pretreatment with Atropine and Cimetidine[1]

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Received 21 March 1983

HENKE, P. G. *Mucosal damage following electrical stimulation of the anterior cingulate cortex and pretreatment with atropine and cimetidine.* PHARMACOL BIOCHEM BEHAV **19**(3) 483–486, 1983.—Bipolar electrical stimulation of a restricted area of the anterior cingulate cortex in anesthetized rats produced stomach erosions. Pretreatment with atropine sulfate prevented the pathological effects of stimulation. Cimetidine was not effective in preventing the gastric damage. It was concluded that the pathological effects of electrical stimulation were cholinergically mediated, whereas, histamine-2 receptors were not involved.

Cingulate cortex Atropine Cimetidine Gastric pathology Mucosal erosions Electrical stimulation

LESIONS and stimulations of various diencephalic and telencephalic limbic structures have produced gastric pathologies in a number of species [13,20]. In the hypothalamus, lesions of the anterior area, tuber cinerium, ventromedial nucleus, and perifornical region have been reported to produce mucosal erosions and ulcers [10, 21, 28, 33]. Similarly, electrical stimulation of anterior and lateral areas in the hypothalamus markedly increased the gastric acidity and also were found to produce gastric erosions and hemorrhages [9,30]. Vagotomy preceeding lateral hypothalamic stimulation prevented these pathological effects in the stomach [26].

Experimental interventions in the telencephalic limbic system also influence gastric functions. Bilateral lesions in the posterolateral amygdala, the bed nucleus of the stria terminalis, the hippocampus, the entorhinal cortex, the prefrontal cortex and the posterior cingulate cortex have been found to increase the stomach pathology produced by physical restraint [14, 16, 18, 19, 25]. On the other hand, lesions in the centromedial amygdala and the anterior cingulate gyrus apparently attenuated the incidence and the severity of stress-induced erosions in rats [16, 17, 19]. Electrical stimulation of the centromedial areas of the amygdala also initiated the development of stomach ulcers in rats and cats [14, 22, 34].

The aim of the present studies was to determine whether electrical stimulation of the anterior cingulate cortex also produces such gastric pathology. Lesions in this region attenuated the pathological effects of immobilization in rats [19] and also altered gastric acid secretions in dogs [31]. Electrical stimulation of this area has been shown to produce various autonomic effects, e.g., blood pressure changes, pyloric peristalsis, and salivation and bladder effects [23,24]. An additional objective was to investigate the effects of prior treatments with atropine and cimetidine on such stimulation-induced gastric effects.

EXPERIMENT 1

Stomach pathology was measured following electrical stimulation in widespread areas of the anterior cingulate cortex of anesthetized rats. The objective was to localize effective stimulating points within this relatively large brain region.

Method

After 24 hr of food deprivation, twenty male Wistar rats, approximately 140 days old, were titrated to a surgical plane of anesthesia, using IP-injections of a mixture of 0.1 g/kg chloralose and 1.0 g/kg urethan. Bipolar electrodes were lowered into either the right or left anterior cingulate cortex (4.0–1.0 mm anterior to bregma, 0.5–1.0 mm lateral to the midline, and at various ventral coordinates from dura). The electrodes were constructed from twisted Formvar-insulated stainless steel wire (0.25 mm dia.). They were connected to a Grass S-88 square-wave stimulator, set at a pulse rate of 50/sec, pulse width of 0.05 msec, and current intensity of 0.2 mA. Stimulation was applied continuously for 4 hr. Following the stimulation session the stomachs were removed. The brains were then extracted after an intra-cardial perfusion using 0.9% saline and 10% formalin.

The brains were embedded in paraffin, cut at 9 μ, and

[1]Supported by grant NSERC A8617.

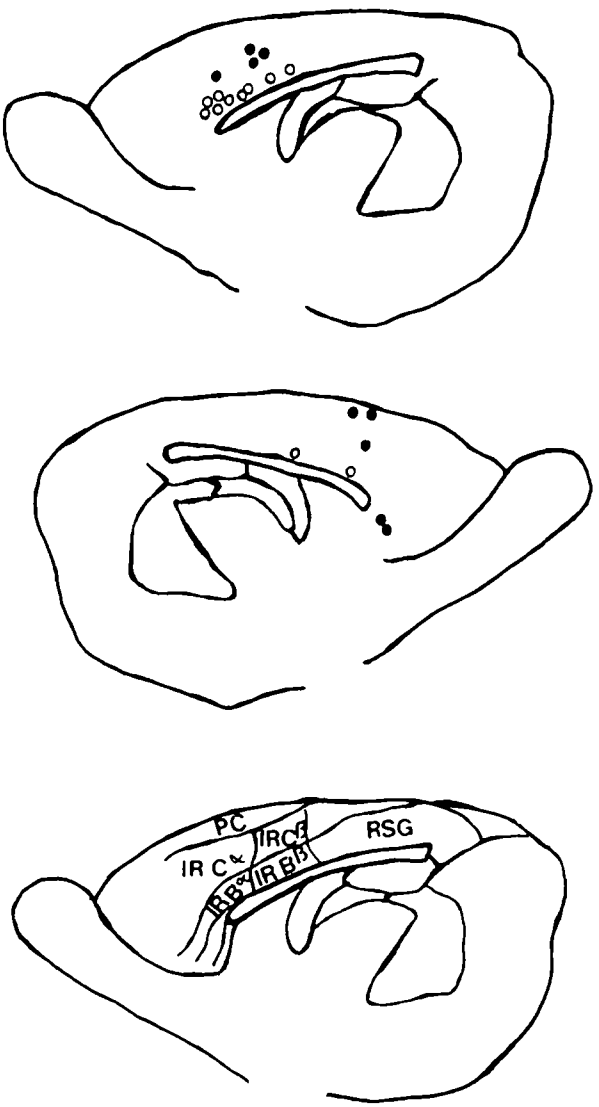

FIG. 1. Effective (open circles) and ineffective (closed circles) stimulation points to induce gastric erosions. Abbreviations: IR (with various subdivisions) = area infraradiata; PC = area precentralis agranularis; RSG - area retrosplenialis granularis (after Domesick [8]).

every tenth section was stained with thionin. The stomachs were opened along the greater curvature, washed in cold water, and examined microscopically. Areas containing erosions were fixed, dehydrated, and embedded in paraffin. Sections were cut at 9 μ and stained with haematoxylin and eosin.

Results and Discussion

Histological examination of the brains showed that the stimulating electrodes had been placed into the anterior midline cortex. Figure 1 indicates that the most effective stimulation points, in terms of stomach pathology, were in the anterior cingulate cortex, immediately dorsal to the corpus callosum. According to the cytoarchitectonic studies of Rose [32] in the mouse, extended to the rat brain by Domesick [8], the most effective stimulating points were primarily located

TABLE 1

GASTRIC PATHOLOGY AFTER STIMULATION IN THE ANTERIOR CINGULATE CORTEX

Subject	Stimulation Site*	Number of Erosions
1 ESAC	IRcβ	0
2	IRcα	0
3	IRcα	0
4	IRcβ	0
5	IRbβ	3
6	IRbα	5
7	PREC	0
8	PREC	0
9	IRa	0
10	IRa	0
11	IRbα	4
12	IRbα	4
13	IRbβ	3
14	IRbβ	6
15	IRcβ	0
16	IRbα	10
17	IRbα	7
18	IRbα	13
19	IRbβ	9
20	IRbβ	2

*Abbreviations: IR (with various subdivisions) = area infraradiata; PREC = area precentralis agranularis (after Domesick [8]).

in areas infraradiata—b α, β. Most of the effective electrodes were near the indusium griseum, and the stria longitudinalis medialis and lateralis. The electrodes that were located in the areas infraradiata—c α, β and the area precentralis agranularis, however, produced no significant pathological effects in the stomachs. Table 1 summarizes the pathology data. Figure 2 shows a photomicrograph of a representative stomach erosion, found in the present study. The mucosal erosions were generally shallow and did not extend to the muscularis layer. All the pathological effects of the stimulation were seen in the glandular portion of the stomachs. Most of the erosions were associated with hemorrhaging in the freshly excised stomachs.

The present results show that gastric pathology can be induced by stimulation of a rather restricted zone in the anterior cingulate cortex. Many of the effective stimulation sites were near the induseum griseum and adjacent to the fibers of the stria longitudinalis medialis and lateralis. However, on the basis of the present data it is not possible to determine to what extent these areas and pathways might be involved in the observed effects in the stomachs. The next study investigated the effects of atropine and cimetidine on the stimulation-induced pathology.

EXPERIMENT 2

Cholinergic blocking agents and histamine-2 receptor blockers have been used extensively in the treatment of stomach ulcers in man, as well as, for the alleviation of stress-induced stomach erosions in experimental animals [2, 3, 4, 11, 12, 29]. In the present study, atropine sulfate (a cholinergic blocker) and cimetidine (a histamine-2 receptor

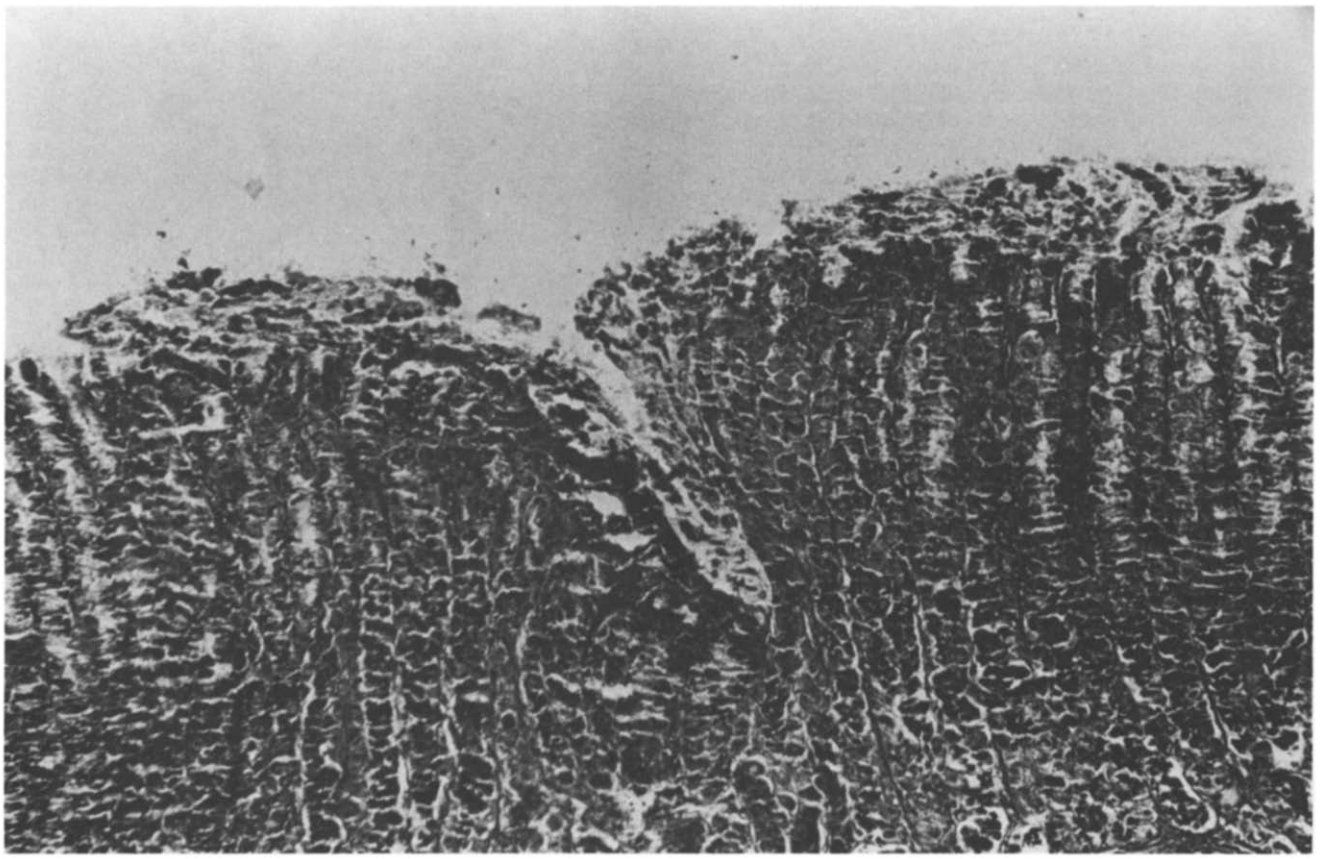

FIG. 2. Photomicrograph of mucosal erosion.

blocker) were administered prior to the elctrical stimulation in the anterior cingulate cortex of rats.

Method

Bipolar stimulating electrodes were lowered into the cingulate region of anesthetized, male Wistar rats, using the procedures describe in Experiment 1. Atropine sulfate (1 mg/kg) and cimetidine (20 mg/kg or 100 mg/kg) were injected subcutaneously using a volume of 5 ml/kg of body weight (n=8). Atropine sulfate was dissolved in 0.9% saline and cimetidine in 0.5 M HCl, (pH=6.0 with 1 M NaOH) in 0.9% saline. Drug administration occurred 30 min prior to brain stimulation. An additional group of rats (n=8) was injected with an equal volume of 0.9% saline prior to stimulation. The stimulation parameters and histological methods were the same as described in Experiment 1.

Results and Discussion

Inspection of the brains showed that the stimulating electrodes were in the anterior cingulate cortex. Most of the electrodes were appropriately placed in areas infraradiata—b α, β, but in two rats of the saline-group and in one rat of the 100 mg/kg cimetidine-group the electrodes were found outside of areas infraradiata—b α, β. These animals were eliminated from the data analysis.

Table 2 presents the data on the stomach pathology in the four groups of rats. It indicates that only cholinergic blockade prevented the pathological effects of electrical stimulation in the cingulate cortex. Cimetidine administration, at dosages of 20 mg/kg or 100 mg/kg, however, produced similar effects as those seen after saline injections. All the cimetidine-injected and saline-injected rats showed some degree of stomach pathology, with the number of erosions ranging from 2–14. These findings suggest that the pathological effects of electrical stimulation of the anterior cingulate cortex, i.e., areas infraradiata—b α, β, are cholinergically transmitted. Apparently, these effects on the stomach do not involve the histamine-2 receptors.

TABLE 2

MUCOSAL DAMAGE FOLLOWING STIMULATION OF THE ANTERIOR CINGULATE CORTEX AND PRETREATMENT WITH ATROPINE AND CIMETIDINE

Treatment	n	Mean Frequency of Erosions
Atropine	8	0*
Cimetidine (20 mg/kg)	8	6.3
Cimetidine (100 mg/kg)	7	5.9
Saline Control	6	6.8

*$p < 0.01$, relative to control (Mann-Whitney, two-tailed).

GENERAL DISCUSSION

The present studies indicate that bipolar electrical stimulation of a rather restricted area of the anterior cingulate cortex of chloralose-urethanized rats produced mucosal erosions. These pathological effects could be blocked with the cholinergic antagonist atropine, but they seemed unaffected by administrations of the histamine-2 receptor blocker cimetidine. These findings suggest that the pathology produced by stimulation in the cingulate cortex involves cholinergic vagal mechanisms, but not the histamine-2 receptor.

Electrical stimulation of the cingulate cortex has been reported to produce a number of autonomic changes in several species. The most pronounced effects of stimulation included blood pressure and heart rate reductions, as well as, respiratory changes, and eating automatisms [1, 7, 23, 24, 27, 35]. Recently, lesions of the anterior cingulate cortex have been found to attenuate restraint-induced stomach erosions in rats, whereas, posterior lesions produced opposite effects [19]. Earlier studies had also shown that massive cingulate damage in dogs altered the acid and pepsin activity, sometimes producing increases and other times decreases [31]. Similarly, clinical data have indicated that surgical lesions in the anterior midline cortex may alleviate ulcers and colitis in human patients [5,6].

REFERENCES

1. Anand, B. K. and S. Dua. Electrical stimulation of the limbic system of brain ("visceral brain") in the waking animals. *Ind J Med Res* **44:** 107–119, 1956.
2. Bonfils, S. and A. Lambling. Psychological factors and psychopharmacological actions in the restraint-induced gastric ulcer. In: *Pathophysiology of Peptic Ulcer*, edited by S. C. Skoryna. Montreal: McGill University Press, 1963.
3. Bonfils, S. and M. Dubrasquet. Psychotropic drugs in experimental peptic ulcer induced by psychological stress. In: *Psychotropic Drugs in Internal Medicine*, edited by A. Pletscher and A. Marino. Amsterdam: Excerpta Medica Foundation, 1969.
4. Brodie, D. A., V. J. Lotti and B. G. Bauer. Drug effects on gastric secretion and stress gastric hemorrhage in the rat. *Dig Dis Sci* **15:** 111–120, 1970.
5. Bucaille, M. Electrocoagulations du cerveau préfrontal. Indications et résultats dans les ulcerès gastrigues et duodenaux rebelles. *Acta gastroenterol Belg* **20:** 506–514, 1957.
6. Bucaille, M. Selective frontal lobe operation for treatment of some diseases of the digestive tract with special reference to ulcerative colitis. *Surgery* **52:** 690–692, 1962.
7. Buchanan, S. L. and D. A. Powell. Cingulate cortex: Its role in Pavlovian conditioning. *J Comp Physiol Psychol* **96:** 755–774, 1982.
8. Domesick, V. B. Projections from the cingulate cortex in the rat. *Brain Res* **12:** 296–320, 1969.
9. Feldman, S. E., A. J. Behar and D. Birnbaum. Gastric lesions following hypothalamic stimulation. *Arch Neurol* **4:** 308–317, 1961.
10. French, J. D., R. W. Porter, E. B. Cavanaugh and R. L. Longmire. Experimental gastroduodenal lesions induced by stimulation of the brain. *Psychosom Med* **19:** 209–220, 1957.
11. Glavin, G. B. Restraint ulcer: History, current reserach and future implications. *Brain Res Bull* **5:** Suppl 1, 51–58, 1980.
12. Hemmer, J., P. O. Schwille, W. Schellerer and W. Hofmann. Effects of cimetidine upon gastric secretion and mucosal blood flow in the rat stressed by restraint. *Res Exp Med (Berl)* **176:** 207–217, 1980.
13. Henke, P. G. The hypothalamus-amygdala axis and experimental gastric ulcers. *Neurosci Biobehav Rev* **3:** 75–82, 1979.
14. Henke, P. G. The amygdala and restraint ulcers in rats. *J Comp Physiol Psychol* **94:** 313–323, 1980.
15. Henke, P. G. The centromedial amygdala and gastric pathology in rats. *Physiol Behav* **25:** 107–112, 1980.
16. Henke, P. G. Facilitation and inhibition of gastric pathology after lesions in the amygdala of rats. *Physiol Behav* **25:** 575–579, 1980.
17. Henke, P. G. Attenuation of shock-induced ulcers after lesions in the medial amygdala. *Physiol Behav* **27:** 143–146, 1981.
18. Henke, P. G., R. J. Savoie and B. M. Callahan. Hippocampal deafferentation and deeferentation and gastric pathology in rats. *Brain Res Bull* **7:** 395–398, 1981.
19. Henke, P. G. and R. J. Savoie. The cingulate cortex and gastric pathology. *Brain Res Bull* **8:** 489–492, 1982.
20. Henke, P. G. The telencephalic limbic system and experimental gastric pathology: A review. *Neurosci Biobehav Rev* **6:** 381–390, 1982.
21. Hoff, E. C. and D. Sheehan. Experimental gastric secretion following hypothalamic lesions in monkeys. *Am J Pathol* **11:** 789–802, 1935.
22. Innes, D. L. and M. F. Tansy. Gastric mucosal ulceration associated with electrochemical stimulation of the limbic brain. *Brain Res Bull* **5:** Suppl 1, 33–36, 1980.
23. Kaada, B. R. Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of "rhinencephalic" and other structures in primates, cat and dog. *Acta Physiol Scand Suppl* **24:** 1–83, 1951.
24. Kaada, B. R. Cingulate, posterior orbital, anterior insular and temporal pole cortex. In: *Handbook of Physiology. Section I: Neurophysiology*, vol 2, edited by J. Field, H. W. Magoun and V. E. Hall. Washington, DC: American Physiological Society, 1960.
25. Kim, C., H. Choi, J. K. Kim, M. S. Kim, H. J. Park, B. T. Ahn and S. H. Kang. Influence of hippocampectomy on gastric ulcer in rats. *Brain Res* **109:** 245–254, 1976.
26. Kurotsu, T., M. Takeda and T. Ban. Studies on the gastrointestinal motility and hemorrhage induced by hypothalamic stimulation in rabbits. *Med J Osaka Univ* **2:** 97–120, 1951.
27. Lofving, B. Cardiovascular adjustments induced from the rostral cingulate gyrus. *Acta Physiol Scand Suppl* **53:** 184, 1961.
28. Luparello, T. J. Restraint and hypothalamic lesions in the production of gastroduodenal erosions in the guinea pig. *J Psychosom Res* **10:** 251–254, 1966.
29. Paré, W. P., G. B. Glavin and G. P. Vincent. Effects of cimetidine on stress ulcer and gastric acid secretion in the rat. *Pharmacol Biochem Behav* **8:** 711–715, 1978.
30. Porter, E., H. J. Movius and J. D. French. Hypothalamic influence on hydrochloric acid secretion of the stomach. *Surgery* **33:** 875–880, 1953.
31. Richter, H. M., R. A. Davis, D. Ruge and N. T. Walter. The effect of cortical and subcortical brain lesions upon gastric secretion in dogs with a vagus-perserved total gastric pouch. In: *Surgical Forum*, vol 3. Chicago: American College of Surgeons, 1957.
32. Rose, M. Cytoarchitektonischer Atlas der Grosshirnrinde der Maus. *J Psychol Neurol (Lpz)* **40:** 1–51, 1929.
33. Schallert, T., I. Q. Wishaw and K. P. Flannigan. Gastric pathology and feeding deficits induced by hypothalamic damage in rats: Effects of lesion type, size and placement. *J Comp Physiol Psychol* **91:** 598–610, 1977.
34. Sen, R. N. and B. K. Anand. Effects of electrical stimulation of the hypothalamus on gastric secretory activity and ulceration. *Ind J Med Res* **45:** 507–513, 1957.
35. Smith, W. K. The results of ablation of the cingular region of the central cortex. *Fed Proc* **3:** 42–43, 1944.