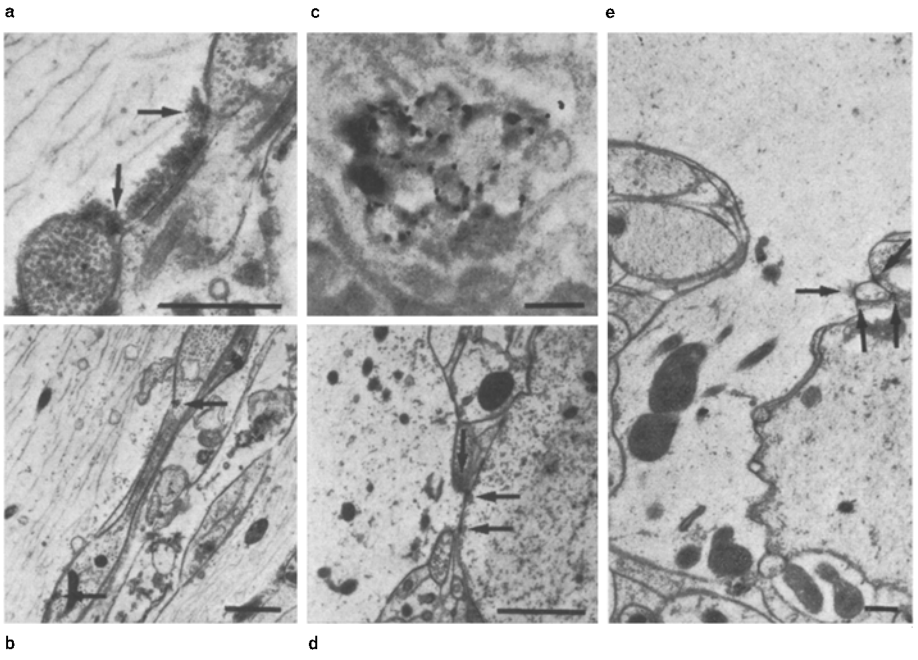

Fig. 2. *a* A giant interneuron is presynaptic to 2 small blebs. 2 divergent diadic synapses are arrowed. The blebs are packed with synaptic vesicles and are themselves presynaptic as well as postsynaptic structures. A transverse section taken at the base of the median ocellar nerve. Bar 0.25 μm indicates magnification. – *b* A number of long blebbed dendrites are presynaptic to giant interneurons which are themselves presynaptic to other small profiles in the same area. 2 divergent diads are arrowed. A longitudinal section from the median ocellar nerve proximal to the brain. Bar 0.5 μm. – *c* A 10 μm wax section through the lateral ocellar tract showing profiles filled by cobalt diffusion from the ventral nerve cord and treated by the modified Timm technique. Long, blebbed dendrites ring the giant interneurons over a considerable length. Thick sections indicate that a number of intrinsic interneurons terminate in a similar way in this region. Bar 10 μm. – *d* 2 giant interneurons are directly apposed over a short length of their membrane, 1 is postsynaptic to the other and also postsynaptic to a smaller profile. In this area a large interneuron may be both pre- and postsynaptic. Input or output synapses are usually grouped together in discrete areas a few microns apart. Here also synapses are usually diadic and divergent. Transverse section of the lateral ocellar nerve in the pars intercerebralis. Bar 1 μm. – *e* The giant interneurons give off very short, stubby collaterals which wrap around other giant second order cells. Small dendrites from third order cells interdigitate repeatedly between them. Synaptic arrangements are generally complex, diadic and reciprocal, black arrows. A transverse section in the brain anterior to the protocerebral bridge. Bar 0.25 μm.

Integration by means of slow potentials in second order cells is a common feature of visual sensory neuropile[10]. In the insect compound eye the large monopolar cells of the lamina only respond to retinal stimulation with graded hyperpolarisations[5,11]. A unique feature in the ocellar system is the size of the second order cells and the distances involved and the fact that the ocellar neuropilar area has become extended over the axonal length of the fibre. A contributory factor here may be the fact that the lateral and median ocelli are linked in pairs by 2 large axons. The axonal integration area described may thus in effect form a common neuropile area for each pair of ocelli. Information processing along visual interneurons of this size and accessibility offers a most promising preparation for examining mechanisms of graded synaptic transmission.

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Modification of the action of pentagastrin on acid secretion by botulinum toxin[1]

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Summary. I.v. botulinum toxin after 60–90 min abolished the dose-response relationship between pentagastrin and gastric acid secretion in anesthetized rats and guinea-pigs. The toxin reduced but did not abolish the acid stimulatory effect of histamine. As expected, the acid response to vagal stimulation was abolished and that to methacholine in rats was unaltered by the toxin.

Vizi et al.[3] have provided evidence that pentagastrin(PG) does not act directly to stimulate guinea-pig intestinal muscle, but via a cholinergic intermediary mechanism. We have found[4,5] that after morphine sulphate or hemicholinium administration to conscious Heidenhain pouch dogs a positive dose-response relationship between i.v. pentagastrin and gastric acid secretion is no longer obtainable. Morphine depresses acetylcholine release at cholinergic neuroeffector sites[6] and hemicholinium interferes with acetylcholine synthesis[7]. This suggests that the action of PG on gastric secretion also requires cholinergic mediation.

Material and methods. As a final test of this hypothesis we have measured PG-stimulated gastric secretion in anesthetized (chloralose) guinea-pigs and rats before and after botulinum toxin. In each animal after anesthesia

the external jugular vein was cannulated and a tracheal tube inserted. A stomach tube was passed and the pylorus cannulated. After completing the preparation of the animal i.v. saline was given for 30 min (3 collections), then 0.04 μg/min pentagastrin until a plateau of acid secretion was reached. At this point 0.5 ng/100 gm of botulinum toxin was given as an i.v. bolus in phosphate buffer at pH 6.8. Pentagastrin was continued for 4 more 10-min periods at 0.04 μg/min. Then it was increased to 0.1, 0.2 and 0.4 for 2 collections at each level. This was succeeded by pentagastrin at a baseline level of 0.1 μg/min for the remainder of the experiment plus histamine, 4 μg/min in guinea-pigs, 0.5 μg/min of methacholine in rats and bilateral vagal stimulation for 20 min in both (2 collections). Before histamine the animals were given 2 mg of promethazine HCl i.v.

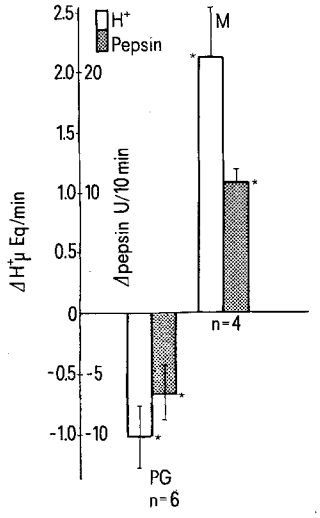

Fig. 1. The effect ± SE of botulinum toxin on pentagastrin-(PG) stimulated acid and pepsin secretion in rats and the effect of methacholine (M) after botulium toxin. PG was given throughout. The bars represent difference from PG baseline (0 line) in the same animal. *significant change from PG baseline, $p < 0.05$. n = number of animals.

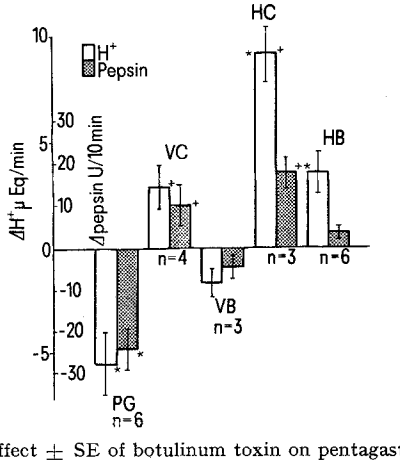

Fig. 2. The effect ± SE of botulinum toxin on pentagastrin-stimulated acid and pepsin secretion and the effect of vagal stimulation and histamine in control guinea-pigs (VC and HC) and in guinea-pigs after botulinum toxin (VB and HB) as in figure 1. PG was given throughout and the bars represent change from the PG baseline (0 line) response in both groups of animals. *significant change from PG baseline, $p < 0.05$. +significant difference from animals given toxin, $p < 0.05$. n = number of animals.

At 10-min intervals the stomachs were flushed with 5 ml of warm saline. These washings were titrated and samples saved for pepsin determination using Anson's[8] hemoglobin method. Controls were conducted in separate animals and duplicated the experiment exactly except that the phosphate buffer did not contain botulinum toxin. Comparison was made both with the change from control in the same animal and collection by collection between control and experimental means using student's t-test for paired and group comparison respectively.

Results. Botulinum toxin brought about a prompt decline to near vanishing point of pentagastrin-stimulated acid and pepsin secretion in rats (figure 1). No subsequent dose response to pentagastrin could be obtained at any of the PG doses tried. Methacholine still produced good secretion despite botulinum toxin. Vagal stimulation was never successful in either control or experimental rats. Substantially the same was seen in guinea-pigs (figure 2). Botulinum toxin abolished the acid and pepsin response to vagal stimulation but not to histamine. Vagal stimulation produced copious secretion in control guinea-pigs. The response to histamine was, however, significantly smaller after toxin than in the control animals. No stimulation of acid and pepsin was obtained with increasing doses of gastrin. The major difference from the rats was in that botulinum toxin itself increased acid secretion in guinea-pigs. This initial burst of secretion gradually declined over 60–90 min. An attractive explanation for this is possible histamine liberation by the toxin in this histamine-sensitive species.

Discussion. Since botulinum toxin exerts its pharmacological action by preventing liberation of acetylcholine from nerve endings, these experiments add further evidence to support the hypothesis that acetylcholine is a necessary intermediate for pentagastrin stimulation of gastric secretion. That the secreting cells are themselves active is evident from the continued efficacy of 2 direct-acting stimulants, histamine and methacholine. Histamine was less effective in guinea-pigs after toxin, but the action of methacholine in rats was unimpaired. Proof that cholinergic secretory mechanisms have indeed been blocked is provided by the failure of vagal stimulation after botulinum toxin to increase acid or pepsin secretion in guinea-pigs. The gastric secretory cholinergic mechanism seems to display a marked sensitivity to botulinum toxin since within the time span of our experiments breathing and skeletal reflex activity remained virtually unimpaired. Similar sensitivity was seen to hemicholinium which, in conscious dogs, produces no obvious impairment other than of gastric secretion[4].

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