EFFECT OF A CARCINOGEN ON ELECTRICAL ACTIVITY OF THE RAT STOMACH

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Acute experiments on rats showed that intragastric injection of an aqueous solution of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) depresses electrical activity of the stomach wall, as reflected in a decrease in the amplitude and frequency of the basic electrical rhythm and, in most cases, its total disappearance. After administration of MNNG the excitatory response of electrical activity both to electrical stimulation of the vagus nerve and to injection of carbachol disappears, but the inhibitory response both to stimulation of the vagus nerve and to injection of ATP is maintained.

KEY WORDS: electrical activity of the stomach; vagus nerve; N-methyl-N'-nitro-N-nitrosoguanidine; carbachol; ATP.

Induction of tumors in the stomach by injection of the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is a convenient experimental model of carcinogenesis because of the ability of MNNG to induce gastric adenocarcinoma selectively in experimental animals in a high percentage of cases [3, 8, 14]. This fact is not only of theoretical, but also of considerable practical interest, because in some countries where the prevalence of carcinoma of the stomach is very high, nitroso compounds are widely distributed in the environment and are also used in food processing. At the same time, it has been shown experimentally that MNNG can be formed from precursors in the stomach, in which it induces an adenocarcinoma [3, 10, 11].

Most investigations into the development of tumors of the stomach in different animals following administration of MNNG have consisted of morphological studies of changes in the gastric mucosa at different stages of the tumor process [3, 10, 13, 14]. From the physiological standpoint, changes in gastric secretion after induction of tumors of the stomach by MNNG have been investigated [8]. As regards the effects of MNNG on electrical activity of the stomach wall, which reflects the state of neuroeffector junctions, this problem has not yet been studied. Relations between the nervous system and tumor development evidently exists and has been a frequent subject for investigation [1, 2, 5, 6]. With the refined electrophysiological methods now available, the balance is tipped in favor of research in this direction [5, 6]. The object of the present investigation was accordingly to study the effect of MNNG on synaptic transmission of nervous impulses in the neuromuscular system of the stomach, as reflected in the electrical activity of its muscular wall.

EXPERIMENTAL METHOD

Experiments were carried out on 25 rats anesthetized with chloralose (80-100 mg/kg, intraperitoneally). After laparotomy, bipolar clip electrodes connected to a UBP2-03 ac amplifier and RPCh2-01 pen recorder, were applied to the serous surface of the pyloric portion of the stomach to record its electrical activity. The vagus nerve, exteriorized on the animal's neck, was placed on stimulating electrodes connected to an ÉSU-1 electronic stimulator. An aqueous solution of MNNG (1.0-2.0 ml) was administered by gastric tube in doses of 2.5-30 mg. The minimal dose corresponded to the daily dose of MNNG given to animals with their drinking water to induce tumors of the stomach [8, 13, 14].

EXPERIMENTAL RESULTS

Electrical activity of the wall of the pyloric portion of the rat stomach consisted mainly of slow waves of potential – the basic electrical rhythm (BER), with a repetition frequency of 4.2 ± 0.4 waves/min, on which

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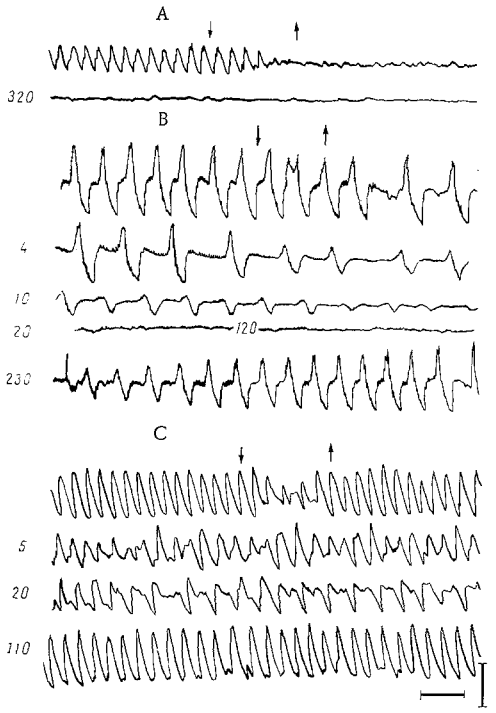

Fig. 1. Inhibition of electrical activity of rat stomach after injection of MNNG. Arrows indicate time of injection of MNNG solution; numbers show time after beginning of MNNG administration when activity was recorded (in min). Calibration: amplitude, 0.5 mV; time, 1 min. Dose of MNNG 15 mg in A and C, 2.5 mg in B.

were superposed fast spike potentials similar to those observed in cats and dogs [4]. After intragastric injection of MNNG solution (starting with 2.5 mg) electrical activity was depressed; the latent period of this response was 0.5-5 min. In most cases (70.5%), the amplitude and repetition frequency of BER were lowered, and the rhythm then disappeared completely: inhibition of BER developed either very rapidly (Fig. 1A), during the first 30-60 sec after injection of MNNG (47%), or it developed gradually (23.5%), and BER disappeared completely after about 20 min. The amplitude and frequency of BER were restored 2-4 h after the beginning of MNNG administration (Fig. 1B), although in 25% of cases BER was not restored even after 6-8 h (Fig. 1A); in such cases complete atony of the stomach could be seen visually.

Sometimes (29.5% of cases) the response to application of MNNG consisted of a change in the shape of BER and a decrease in its repetition frequency from 4.2 ± 0.4 to 1.9 ± 0.2 waves/min ($P < 0.001$); disappearance of BER was not observed in such cases (Fig. 1C). Under these circumstances the amplitude and frequency of BER were restored 1-3 h after the beginning of rinsing the gastric mucosa to remove MNNG (Fig. 1C). Control injection of Ringer's solutions or distilled water into the lumen of the stomach in the same volume and at the same speed as the MNNG solution had no effect on electrical activity.

In the present experiments, no direct correlation was found between the intensity of the response, its latent period, and its duration, on the one hand, and the dose of MNNG on the other hand. The absence of such correlation can be explained by the presence of a glucoprotein barrier in the stomach, which interferes with contact between the MNNG solution and the mucosa [14]. Indirect support for this explanation is given by the fact that the inhibitory effect on electrical activity after administration of MNNG was observed in the present experiments more often and after a shorter latent period in satiated animals than in hungry animals.

The facts described above thus indicate that MNNG has an inhibitory effect on electrical activity of the gastric wall, reflected in depression of the BER. The next step was accordingly to study the mechanism of the blocking action of MNNG on synaptic transmission influences from the vagus nerves of the stomach.

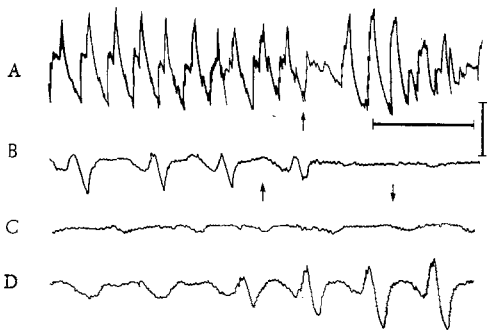

Fig. 2. Effect of MNNG (2.5 mg) on response of gastric electrical activity to vagus nerve stimulation. Period of stimulation indicated by arrows. A) Inhibitory-excitatory response to vagus nerve stimulation (1.5 V, 10 Hz, 0.5 msec) before injection of MNNG; B-D) inhibitory response to vagus nerve stimulation 10, 15, and 20 min respectively.

The vagus nerves are known to contain excitatory and inhibitory fibers to the stomach [4]. In the present experiments electrical stimulation of the vagus nerves evoked either an excitatory effect on electrical activity, with an increase in the amplitude of the slow component of BER and the appearance of spike potentials, or an inhibitory response of depression of BER (or even its complete disappearance), or a mixed effect (inhibition–excitation or vice versa) as a result of coincidence of the thresholds of activation of the excitatory and inhibitory fibers of the vagus nerves, as previous experiments on cats and dogs have demonstrated [4]. An example of such a mixed inhibitory–excitatory response to electrical stimulation of the vagus nerves is given in Fig. 2. After administration of MNNG in the present experiments an excitatory response to vagus nerve stimulation was never observed; the inhibitory effect, however, was preserved or even enhanced, and it became more prolonged, despite the cessation of stimulation (Fig. 2B-D). Inhibition in response to vagus stimulation, it will be noted, was observed after administration of MNNG in cases when the inhibitory effect of MNNG had not yet reached an adequate level, i.e., when BER was still preserved.

The excitatory parasympathetic mediator for electrogenic gastric motor activity is acetylcholine. In the present experiments an excitatory effect, namely the appearance of strong spike activity (Fig. 3A), was evoked by intravenous injection of the cholinomimetic drug carbachol (0.5 ml of a 0.01% solution); its action is more prolonged than that of acetylcholine of MNNG, carbachol was found to be almost ineffective: its action was very short and weak (Fig. 3B). It can tentatively be suggested that MNNG has a blocking effect on synaptic transmission in the parasympathetic excitatory pathway for regulation of electrogenic gastric motor activity.

It has recently been shown that MNNG reduces the transmural potential difference recorded from the gastric wall in dogs and inhibits active Na^+ and Cl^- transport [12].

Meanwhile, MNNG did not depress inhibitory responses of gastric electrical activity to activation of inhibitory fibers of the vagus nerve. Since the mediator for nonadrenergic inhibitory influences is known to be ATP or a related nucleotide [9], in the present experiment ATP was injected intravenously (1 mg/kg), after which an inhibitory response was recorded as a decrease in the amplitude and frequency of BER (Fig. 3C) or even its complete disappearance. This effect was like the inhibition evoked by vagus nerve stimulation. After injection of MNNG the inhibitory response to injection of ATP not only was preserved, but its duration was actually increased (Fig. 3D), just as was observed during vagus nerve stimulation after administration of MNNG.

There is evidence that the inhibitory effects of ATP are connected with changes in membrane permeability for Ca^{++}, for in calcium-free solutions the inhibitory effect of vagus nerve stimulation on motor activity of the rat stomach was reversed to excitatory [7].

As regards the mechanism of action of MNNG recent work has shown that it causes methylation of nucleic acids and acts on proteins, producing nitroamination of lysine residues [14]. However, the fundamental cause of the carcinogenic effect of MNNG has not yet been discovered.

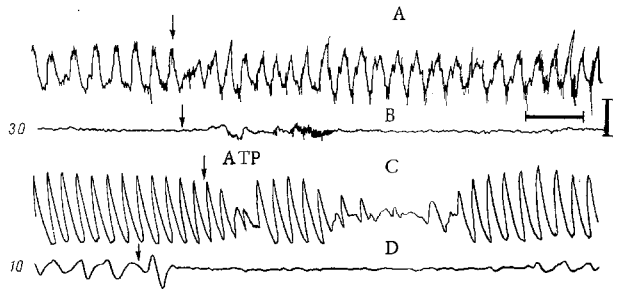

Fig. 3. Effects of intravenous injection of solutions of carbachol and ATP before (A and C) and after (B and D) administration of MNNG (15 mg). Arrows indicate time of intravenous injection of substance. Remainder of legend as in Fig. 1.

The results of the present investigation show that MNNG has an inhibitory effect on electrical activity of the stomach wall, reflected as depression of BER. It can be postulated that MNNG blocks the transmission of excitation in parasympathetic fibers, but the mechanism of inhibition produced by MNNG evidently differs from the mechanism of nonadrenergic inhibition. The experimental results show that even in the earliest stages of contact between the carcinogen and the gastric mucosa substantial disturbances of synaptic transmission of excitation are found. These disturbances precede the carcinogenic process and, by impairing the nutrition of the stomach tissues, they may create favorable conditions for malignant change.

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