Biomedical Research 2003; 14 (1):75-79
Special issue: Autonomic Nervous System

Dedicated to Professor Geoffrey Burnstock

The *in vivo* effect of intravenous ATP on the activity of smooth muscles in the canine, rat and human stomach and intestine

[1]L. Kasakov[1] and M. Vlaskovska[2]

[1]Institute of Physiology, Bulgarian Academy of Sciences, Bonchev street, bldg. 23, 1113 Sofia, Bulgaria
[2]Department of Pharmacology and Toxicology, Medical University Sofia, 2, Zdrave street, 1431 Sofia, Bulgaria

The concepts transform the information into knowledge
C. McBrooks [1]

Introduction

Three decades ago Geoff Burnstock introduced the concept of purinergic neurotransmission executed through purinergic nerves and receptors. The concept ignited ever-growing research activities aimed to prove or disprove the transmittory role of ATP. The physiological significance of purinergic neurotransmission is now firmly established and most scientists consider the information of transmittory action of purines as valuable scientific knowledge. We present here studies that have been greatly stimulated by the purinergic concept and were carried out shortly after the appearance of Burnstock's classical review entitle The purinergic nerves [1]. Investigations were aimed to examine the effect of intravenous ATP on motor and electric activities of canine, rat and human gastrointestinal smooth muscles. The results have been partially presented earlier [3-6].

Methodology

The experiments were conducted in accordance with the Declaration of Helsinki [(7] and the regulations of the local ethics committee. *(i*) Recording of the motor and electric activities of stomach and intestine in dogs. Prior to experimentation macroelectrodes were implanted subserously in the stomach and intestine to record the electric activity [8] and artificial fistula of stomach or jejunum was prepared for volumetric recording of motor activity of stomach and intestine [9] in female dogs (12-15 kg). Dogs recuperated for 2 weeks after surgery and were catheterized through middle elbow vein receiving local skin anesthesia before examination. ATP (Atriphos, Gedeon Richter, Hungary) was injected in 60s through the catheter in unanesthetized animals at doses 2-4mg/0.1ml/kg. Motor and electric activities were recorded continuously in unrestrained dogs for 30 min after injection of equal volume saline and 60 min after ATP injection. *(ii) X-ray examination of gastrointestinal tract in rats.* Prior to examination male rats (Wistar, 250-300 g) were catheterized through *dorsal penile vein* under ether anesthesia and placed on warmed plastic table. Gastrographin (Schering, Germany) was applied in volume 0.2 ml/100g during stomach catheterization and three X-ray pictures were taken at 5min intervals. ATP (Atriphos, Gedeon Richter, Hungary) was injected in 60s through the catheter at dose 1.0mg/0.1ml/100g. Stomach and intestine were x-rayed 1, 2, 3, 5, 7, 10, 15, 20, 30, 60 min, and 2, 3, 4 and 5h after ATP application. *(iii) X-ray examination of gastrointestinal tract of healthy volunteers.* Five volunteers in sound physical and mental health (3men, aged 30, 32 and 38 years, 63.4±3.7kg body weight, 1.62±0.26sqm body surface, and 2 women, aged 27 and 30 years, 49.7±3.1kg body weight, 1.22±0.19sqm body surface) entered the study with signed consent. Blood pressure and heart rate were monitored. Prior to examination *middle elbow vein* was catheterized. The volunteers drank then 200ml Gastrographin (Schering, Germany) and two X-ray pictures were taken at 30min intervals after injection of equal volume saline. ATP (Atriphos, Gedeon Richter, Hungary) was injected in 60s through the catheter at dose 1.0mg/0.1ml/kg. Stomach and intestine were X-rayed 1, 2, 3, 5, 7, 10, 15, 20, 30, 60 min, and 2, 3, 4, 5, 6, 12 and 24h after ATP application. The equipment was Philips-Muller DA701 roentgenograph with monitoring system and cinematograph/seriograph. The X-ray parameters were: voltage 45kV (rat) or 75-90kV (human), exposure time 0.06s (rat) or 0.12-0.15s (human) and focus-to-film distance 60cm (rat) or 70-80cm (human).

Results and Discussion

I. The effect of ATP on motor and electric activities of stomach and intestine in dogs

ATP inhibited the tonic and phasic contractions of stomach and intestine, which was accompanied by disappearance of spikes in electromyograms. The drug evoked strong relaxation of stomach and intestine, which reached

maximum in seconds and lasted for several minutes after injection. ATP decreased the frequency of myoelectric complex (BER) in stomach with a little change (9-15%) of propagation rate (Fig. 1). In contrast ATP increased BER propagation rate in intestine (27±4%) without change in frequency. Bradycardia with bigeminy occurred during ATP injection and continued 60-90min. (ii) X-ray examination of gastrointestinal tract in rats. ATP evoked rapid relaxation of the GIT. The gastric image enlarged and duodenal arch and intestinal loops relaxed (Fig. 2). The peristalsis was less efficient which culminated in motor standstill of duodenum. The effect was less in distal segments of intestine. ATP evoked relaxation and atonia of the colon followed at later stages by changed mucosal hydrodynamic balance and flatulency. The motor activity normalized slowly after 3-4h. *(iii) X-ray examination of gastrointestinal tract of healthy volunteers.* ATP evoked rapid relaxation of stomach and duodenum in the first 60s that reached maximum after 10-12min (Fig. 3). The stomach extended downwards and bottom of gastric image projected 4-5cm beneath the iliac line. Due to less efficient and weak peristalsis contrast material remained in duodenal bulb longer time, which is a characteristic feature of gastric hypotonicity or pyloric stenosis. The effect of ATP was less in distal segments of small intestine. The normal motor activity of stomach and small intestine returned after 2-3h. The systolic blood pressure decreased in the first 30min by 21± 4% accompanied by moderate bradycardia.

The results showed that intravenous ATP caused rapid and sustained relaxation of gastric and intestine smooth muscles with specific pattern in the electromyogram, i.e. decrease of BER frequency in the stomach and increase of BER propagation rate in small intestine. This was likely a specific effect since intravenous noradrenaline, atropine or papaverine increased BER frequency. Gastric relaxation with same electrophysiological correlates was produced by efferent vagal stimulation in presence of atropine, which suggested that the effect was realized via parasympathetic non-adrenergic, non-cholinergic inhibitory nerves [10] supplying stomach pacemaker area [11]. The results provide first evidence from X-ray examination that intravenous ATP caused fast relaxation of human stomach and intestine. The hypotensive and bradycardic effect of ATP might be attributed to its negative chronotropic and vasodilatory action [12]. Present study showed that in rat intravenous ATP produced relaxation of whole gastrointestinal tract including colon. The results did not verify whether ATP or other substance (ATP derivative or ATP released) produced relaxation of gastrointestinal smooth muscles. Unless specific ATP antagonists were available it could only be speculated that relaxatory action of ATP is mediated through specific purinoceptors.

Fig. 1: The effect of Atriphos on motor and electric activities of canine stomach

The upper panel shows original electrogastrograms recorded by two adjacent electrodes (1, 2). Lower panel shows volumetric registration of stomach motility of the same dog. Middle panel represents BER frequency (mean ± sem) during period designated by dashed lines. The data are representative of all experiments (6-8 experiments in each of 9 dogs).

Fig. 2: *X-ray photographs of gastrointestinal tract in rat. Clockwise the pictures show X-ray images before and after ATP injection. Time after ATP injection is shown on each panel. The figure is representative of X-ray examination of all animals (8 rats).*

Fig. 3: *X-ray photographs of stomach and duodenum in human. Clockwise the pictures show X-ray images before and 1, 3 and 10min after ATP injection. The figure is representative of X-ray examination of gastrointestinal tract of all volunteers (3 men and 2 women).*

References

1 Brooks C.McC. Newer concepts of the autonomic system's role derived from reductionist and behave ioral studies of various animal species. J Autonom Nerv Syst 1983; 7: 199-212.

2. Burnstock G. Purinergic nerves. Pharmacol Rev 1972; 24: 509-581.

3. Kasakov L. Comparative studies on ATP and Oxytocin inhibitory actions on gastro- intestinal smooth muscles. Proceedings of 1st International Symposium Physiology and Pharmacology of Smooth Muscle, R32. Varna, 28-30 September 1976.

4. Kasakov L. The effect of Oxytocin, ACTH and Insulin on bioelectrical and mechanical activities of stomach and intestine. Ph. D. thesis (Medicine) Sofia 1979.

5. Kasakov L, Milenov KM. Vlaskovska. Myoelectrical patterns of "purinergic" gastric relaxation. Proceedings of 2nd International Symposium Physiology and Pharmacology of Smooth Muscle, R41. Varna, 09-11 October 1976.

6. Kasakov L, Tomov AM. Vlaskovska. On the effect of Adenosine triphosphate (Atriphos) on the motor activity of the gastrointestinal tract. Exp Med Morphol 1988; 4: 9-11.

7. Declaration of Helsinki: Human experimentation. Code of ethics of the World Medical Association. Br Med J; 18 July 1964.

8. Jansson G, Martinson J. Some quantitative considerations on vagally induced relaxation of the gastric smooth muscle in the cat. Acta Physiol Scand 1965; 63: 351-357.

9. Papasova M, Milenov K. Method for registration of stomach biopotentials in cat by chronically implanted electrodes. Proc Inst Physiol 1965; 9: 187-191.

10. Kasakov L. Physiology of purinergic transmission. D. Sc. thesis (Medicine), Sofia, 1989.

11. Martinson J. Studies on the efferent vagal control of the stomach. Acta Physiol Scand 1965; suppl 255.

12. Fukuda AF, Flacke UE, Bloor BC. Hypotensive effects of adenosine and adenosine triphosphate com pared to sodium nitroprusside. Anest Analg 1982; 61: 273-278.

Correspondence to:

Professor L. Kasakov
Institute of Physiology
Bulgarian Academy of Sciences
Bonchev Street, Blding. 23
1113 Sofia, Bulgaria

e-mail: kasakov@iph.bio.bas.bg

Fig. 3: X-ray photographs of stomach and duodenum in human.
Clockwise the pictures show X-ray images before and 1, 3 and 10min after ATP injection. The figure is representative of X-ray examination of gastrointestinal tract of 5 all volunteers (3 men and 2 women).