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SYSTEMIC EFFECTS OF ADENOSINE TRIPHOSPHATE

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The experiments described in the present paper were the outcome of an incidental observation made in decerebrate cats when adenosine triphosphate (ATP) was injected into the artery supplying a leg muscle, the tibialis anticus. These injections were intended to produce the direct muscle contracting action of ATP first described by Buchthal and Kahlson (1944). We started with small doses of ATP which gave no contractile response; the dose was then increased with the result that sometimes, but by no means always, immediate weak contractions could be recorded from the tibialis anticus, but the injections produced additional and more striking effects. After a latency of some 30 seconds, the time necessary for the ATP to reach the general circulation after having passed through the tibialis anticus muscle, respiratory changes occurred, followed by strong contractions of the skeletal muscles of the limbs and the whole body of the animal. ATP is known to produce profound changes in the cardio-vascular system (Drury and Szent-Györgyi, 1929; Gaddum and Holtz, 1933; Gillespie, 1934; McDowall, 1944; Bielschowsky, Green and Stoner, 1946) and the possibility was therefore envisaged that these general effects might be the outcome of circulatory events.

The present paper deals with circulatory, respiratory, and other systemic effects of ATP when injected into different parts of the circulatory system.

METHODS

Most experiments were performed on cats, decerebrate or in chloralose anaesthesia; a few experiments were performed on dogs and rabbits also anaesthetized with chloralose. The systemic arterial blood pressure was recorded from the left external carotid or the femoral artery with a mercury manometer. In the same way the pressure in the pulmonary artery was recorded in some cats as well, the artery of the lower lobe on the left lung being cannulated in this case. The heart rate was read from the tracing of the systemic blood pressure; for this purpose the drum was run at a fast speed.

For intravenous injections of ATP cannulae were tied into a jugular or femoral vein. When the ATP was intended to reach the systemic, without first passing the pulmonary circulation, it was injected either into the left auricle or through a glass cannula tied into the left auricle or through a long fine syringe needle, with a blunt tip, which was passed through an opening in the right external carotid or subclavian artery down to the base of the ascending aorta. Heparin was often given in these experiments. For injecting ATP into the brain circulation it was injected into the vertebral or external carotid artery. For the injections into the vertebral artery again a fine blunt needle attached to a syringe, filled with the amount of ATP to be injected, was passed through an arterial side branch into the vertebral artery. In order to ensure that all the injected ATP passed down the vertebral artery the vessel was sometimes tied over the needle during the injection, but immediately afterwards the ligature was loosened and the needle withdrawn so that the blood supply to the vertebral artery was restored. When ATP was injected into the external carotid, the carotid sinus region was usually denervated and the injections were made through a cannula tied into the lingual artery pointing toward the carotid, which during the injections was kept clamped at its aortic end with an arterial clip.

When we began injecting ATP into the left coronary artery we used cats and introduced a fine blunt syringe needle, slightly bent at its end, through an opening in the subclavian artery into the ascending aorta and then guiding it by touch into the mouth of the left coronary artery. It was either kept here without further fixation during the injection or tied with a thread previously placed loosely round the origin of the coronary artery. In these experiments the left coronary artery is supplied with blood by collaterals only. Later on we used, in cats and dogs, the methods described by Dawes for cats (1947). With this method the circulation through the left coronary artery is not interrupted. A specially shaped glass cannula is required for these experiments; it was kindly supplied to us by Dr. G. S.

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Dawes (Oxford). In all experiments with injections into the left coronary artery heparin was used.

Respiration was recorded by a modification of Gaddum's (1941) method. The trachea was attached to a glass tube with inflow and outflow valves, which gave as little resistance to respiration as possible. The expired air was blown into a 10 litre bottle, from which a fine glass tube led to the outside air and a wide tube to a sensitive tambour for recording the respiratory changes. Unlike Gaddum's method this arrangement does not provide a quantitative record of the respiratory changes, but it serves the purpose of recording easily changes in rate and depth of respiration. Inspiration is shown in the tracings as a downward, expiration as an upward stroke.

In some experiments it was desired to exclude vagal impulses for short periods. For this purpose the vagi were cooled at the neck by placing them each on a separate 1.5 cm. wide thin copper plate, which was slightly bent at its end in order to prevent the nerves from slipping off the plate. The plates were 16 cm. long and kept cold during the period of cooling the nerves by placing the free ends into beakers full of crushed ice. In addition a little metal box was soldered on each plate near the end provided to take the nerve and also filled with crushed ice.

Three different preparations of the barium salt of ATP were used. One sample was prepared by ourselves by a modified version of Lohmann's method (1931), one sample was kindly supplied to us by Professor F. Buchthal (Copenhagen), and a third sample was obtained from Boots' Drug House. Before use the Ba salt was converted into the sodium salt. When the three preparations were compared for their ability to activate the synthesis of acetylcholine in extracts of acetone dried brain tissue the sample obtained from Boots was found to be only 80 per cent as active as the other two samples, which had practically identical activity. No obvious difference was observed with the three preparations on injections into the circulations of cats or dogs; a 20 per cent reduction in activity, however, would not have been noticed in these experiments. In the text the dose of ATP injected has been expressed as ATP pyro—P (ATP—P). In order to obtain the value for the total phosphorus injected the value has to be multiplied by 1.5. In a few experiments control injections were made with creatine phosphate which was kindly prepared for us by Dr. P. Eggleton and Dr. Nimmo Smith as the synthetic Ba salt and converted before use into the Na salt. The values for creatine phosphate are expressed in the text as mg. total labile phosphorus (CrP—P), about 1 mg. of which is present in 12 mg. of the Ba salt.

RESULTS

Circulatory effects in cats

The intravenous injection of 0.2 to 0.4 mg. of ATP—P causes a profound and steep fall in arterial blood pressure (Fig. 1). During the fall the heart pulsations on the blood pressure tracing become feeble or the float on the mercury manometer may even write a smooth line on the smoked drum. When the oscillations are not obliterated, pronounced slowing of the heart can be seen on the blood pressure tracings. In Fig. 1a the steep fall in arterial blood pressure is associated with an initial period in which the heart oscillations are no longer visible on the tracing; later pronounced slowing of the heart will be seen. In the experiment of Fig. 1b the blood pressure tracing gives during the whole period of the depression a smooth line.

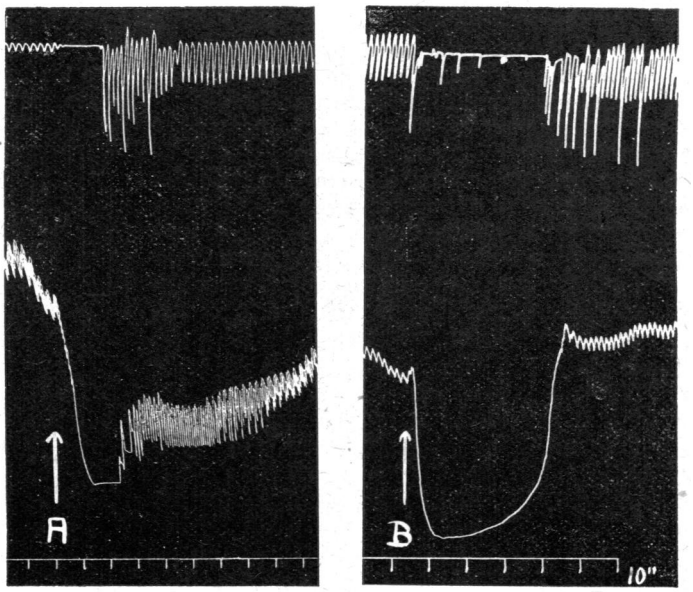
When the blood pressure has recovered a renewed injection of ATP produces approximately the same strong fall in arterial blood pressure as the first injection, but on further repetition of the injections the sensitivity to ATP gradually decreases, so that it has to be given in increasing doses in order to elicit responses as strong as the initial ones.

Other organic as well as inorganic phosphate compounds injected in doses containing the same or even a greater amount of total phosphate than that injected with the ATP, produced either no fall in arterial blood pressure (for instance 0.6 mg. CrP—P, 7 mg. sodium triphosphate or 15 mg. sodium pyrophosphate) or a fall uncomplicated by slowing of the heart or by reduction in the heart oscillations (for instance 10 mg. muscle or yeast adenylic acid).

The following factors are responsible for the depressor action of ATP in cats: (*a*) obstruction in the pulmonary circulation, causing a reduction in cardiac output, (*b*) reflex slowing of the heart causing further reduction in cardiac output, and (*c*) vasodilatation.

Obstruction in the pulmonary circulation.—In order to observe the effect of ATP on the pulmonary circulation it is best either to cut the vagi or to give atropine in order to eliminate the slowing of the heart. Under these circumstances ATP still produces its pronounced depressor effect, showing that the slowing of the heart observed in the absence of atropine and with the vagi intact is not the sole or main factor responsible for the fall in systemic blood pressure produced by ATP.

The injection of 0.2 to 0.4 mg. ATP—P into the jugular vein or into the right auricle of a cat with the thorax open, artificial ventilation and the vagi cut or atropine given, produces tremendous swelling of the pulmonary artery and of the whole right heart, ventricle and auricle; the left remains small. This can be observed with the naked eye in a cat with open thorax and artificial ventilation. These volume changes do not take place when the



(a) (b)

FIG. 1.—Respiration (upper) and arterial blood pressure (lower) tracings of decerebrate cat (*a*) and cat under chloralose (*b*). At A, 0.2 mg., at B, 0.4 mg. ATP–P intravenously. Time in 10 seconds.

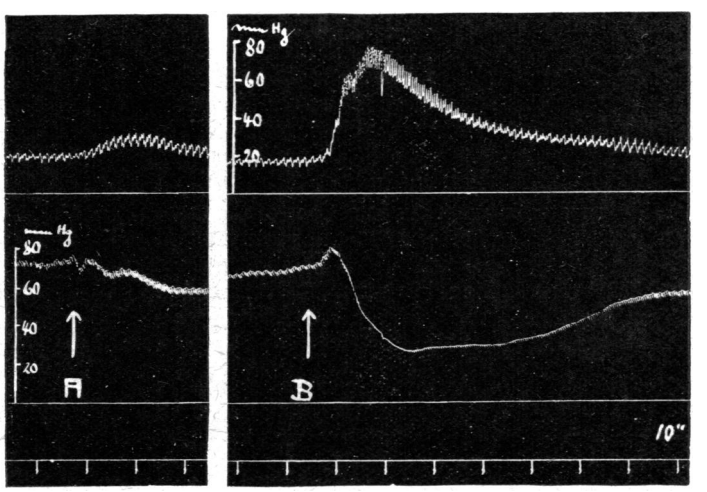


FIG. 2.—Pressure in pulmonary artery (upper tracing) and carotid (lower tracing) of 2.2 kg. cat under chloralose. Thorax opened ; artificial ventilation ; both vagi cut. At the arrows 0.2 mg. ATP–P into the left auricle (at A) and into the jugular vein (at B). Time in 10 seconds.

same dose of ATP is injected into the left auricle and reaches the pulmonary artery greatly diluted after its passage through the systemic circulation. Incisions into the carotid arteries, when the intravenous injection of ATP had produced its characteristic fall in arterial blood pressure with elimination of the heart oscillations and the typical changes in heart volume, caused very little bleeding, whereas a subsequent incision into the right auricle or the superior vena cava produced a rushing out of blood into the thorax. All these observations are accounted for by a strong obstruction in the pulmonary circulation whereby the blood is prevented from reaching the left heart; consequently the cardiac output drops.

In Fig. 2 the pulmonary obstruction produced by ATP is shown by recording its effect on the pressure in the pulmonary artery simultaneously with the carotid blood pressure. Usually the opening of the thorax and tying a cannula into a pulmonary artery and into the left auricle lowers the carotid blood pressure to about 100 mm. Hg or to an even lower level. In this condition ATP apparently produces little further vasodilatation, as seen from the fact that an injection of 0.2 mg. ATP—P into the left auricle causes scarcely any further fall in carotid blood pressure (Fig. 2,A). The pressure in the pulmonary artery also is little affected by this injection of ATP. The result, however, is different when the same dose of ATP is injected intravenously (at B); the pressure in the pulmonary artery rises immediately and steeply from its initial level of about 20 mm. Hg to about 80 mm. Hg. The pressure in the carotid artery starts to fall a second or two after the beginning of the rise in the pulmonary artery and then follows it in reverse direction. During the fall in systemic blood pressure the heart oscillations on the carotid pressure tracing become feeble.

With repeated intravenous injections of the same dose of ATP the effect on the pulmonary blood pressure becomes gradually smaller and so does the fall in systemic blood pressure. But an increase in the dose of ATP produces again the strong pressure changes in the pulmonary and carotid artery.

Vasodilatation in the systemic circulation.—We have seen that in cats in which a cannula has been tied into the pulmonary artery the arterial blood pressure often falls to a level of about 100 mm. Hg or lower owing most likely to a decrease of tone in the systemic vessels. In such cats no evidence could be obtained for a vasodilator action of ATP. The result, however, is different in those cats in which the level of the arterial blood pressure has remained high even after opening the thorax and tying a cannula into the left auricle; in these conditions ATP in doses too small to produce a fall in arterial blood pressure, when injected intravenously, has such an action when injected into the left auricle. In the experiment of Fig. 3 a dose of 0.05 mg. ATP – P injected intravenously

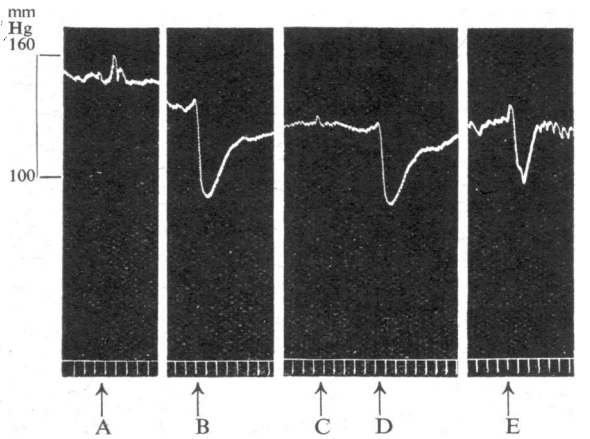


FIG. 3.—Carotid blood pressure of 3.2 kg. cat under chloralose. Both vagi cut; thorax opened, artificial ventilation. At A and E intravenous injection of 0.05 mg. and 0.2 mg. ATP–P respectively. At B and D 0.025 mg. ATP–P and at C 1 c.c. saline injected into the left auricle. Time in 10 seconds.

at A did not lower the arterial blood pressure, but half the amount injected into the left auricle, at B and D, caused an evanescent but pronounced fall, which in fact was stronger than that produced by eight times the dose of ATP injected intravenously at E. Vasodilatation in the systemic circulation therefore also contributes to the usual depressor action of intravenous injections of ATP.

No experiments have been carried out to analyse the vasodilatation. It is unlikely that reflex vasodilatation occurs with ATP when the vagi nerves have been cut. This may happen, however, in cats with intact vagi which could carry afferent impulses to the vasomotor centre. The effect would then resemble that of veratrin (Dawes, 1947). Our experiments also do not deal with the possibility of a centrally induced vasodilatation. Such an effect cannot be excluded by our finding that ATP, at least in cats under chloralose anaesthesia, has no central cardiac action.

Slowing of the heart.—The pronounced slowing of the heart when ATP is injected intravenously is dependent on the integrity of the vagi. This had been observed by McDowall and by Bielschowsky, Green, and Stoner (1946). They had concluded that ATP stimulates the vagus centre in the medulla.

Our results, however, show that the slowing is mainly accounted for by a reflex, the afferent and efferent impulses of which are carried in the vagus nerve. In fact in cats under chloralose anaesthesia the bradycardia obtained on injections of 0.2–0.4 mg. ATP – P is solely accounted for in this way. In decerebrate cats, on the other hand, stimulation of the cardio-inhibitor centre by ATP probably contributes to the bradycardia. In these cats injections of 0.2 to 0.4 mg. ATP – P into a vertebral artery slow the heart, but in cats under chloralose neither an injection into a carotid artery, in central direction, nor into a vertebral artery was effective, whereas the same amount of ATP injected into the left auricle produced pronounced bradycardia even when the carotid and vertebral arteries were occluded during the injection. It might be mentioned in this connexion that clamping of the vertebral arteries in decerebrate cats, but not in cats under chloralose, also led to bradycardia. The reflex nature of ATP-bradycardia in cats under chloralose anaesthesia is illustrated by the experiment (Fig. 4) in which 0.1 mg. ATP – P was injected either into the right vertebral artery (at B) or into the left auricle (at A). The injection into the auricle reduced the heart rate from 34 per 10 seconds to 9 per 10 seconds, whereas the vertebral injection did not change it. Between B and C the carotid and vertebral arteries were clamped ; this caused a rise in blood pressure but no slowing of the heart. When the blood pressure had reached a relatively steady level 0.1 mg. ATP – P was again injected into the left auricle, at C. The bradycardia was not prevented by having excluded the brain circulation, but was abolished after cutting the vagi. In another experiment on a cat in chloralose anaesthesia 0.1 mg. ATP – P was injected into the right carotid, whilst it was kept clamped for a few seconds at its aortic end, or into the left auricle, the carotid and vertebral arteries being kept occluded for about 10 seconds before and after the injection. Again only the injection into the left auricle caused slowing of the heart (from 3.4 to 1.5 beats per second) despite the fact that the pathway to the brain was occluded, but the bradycardia was abolished after cutting the vagi. This experiment shows in addition that the reflex does not originate from chemoreceptors in the carotid body, a fact which has been verified in cats with both carotid sinus nerves ligated and cut. These experiments do not exclude an action of ATP on the chemoreceptors in the aortic body, but it would be strange if they were, and those in the carotid body were not, sensitive to ATP. The region of the aortic arch, however, has not been excluded as a contributory area from which the reflex could be elicited although, to some extent at least, it originates in the heart itself and thus resembles the bradycardia produced by veratrin alkaloids (Dawes, 1947) ; this was evident when ATP was injected into the coronary artery.

In one cat we succeeded in tying a needle cannula, introduced through the subclavian artery, into the main branch of the left coronary artery without causing ventricular fibrillation. Fig. 5 is taken from this experiment ; the injection of 0.5 c.c. of saline into the coronary artery had no effect (at A), but 0.1 mg. ATP – P slowed the heart rate from 28 per 10 seconds to 13 per 10 seconds. The effect of ATP could be obtained with each renewed injection, but only as long as the vagi were left intact. A post mortem injection of Chinese ink through the cannula into the coronary showed that it supplied practically the whole left heart. There must, however, have been effective anastomoses with other coronary branches which kept the muscle supplied with blood. Some indication of these was obtained during the experiment by the fact that there was a strong back flow of blood whenever the syringe attached to the needle was removed after an injection. In later experiments Dawes's method was used in which the coronaries are continuously supplied with blood. In these experiments also the injections of ATP into the coronary circulation caused slowing of the heart, but not after cutting the vagi. Doses larger than 0.2 mg. ATP – P were not tried. The slowing produced with the vagi intact was often less pronounced than that following the injection of a similar dose of ATP low down into the ascending aorta. This difference may be due to the fact that only part of the heart area from which the reflex is elicited will be reached when ATP is injected through the cannula tied into one coronary or that some receptors for this reflex are situated in the aortic arch.

Slowing of the heart by ATP in rabbits and dogs. —Our results obtained in *rabbits* are in agreement with those of Bielschowsky *et al.* (1946) who found that in these animals ATP has a direct cardiac depressant action. The injection of ATP into the left auricle caused pronounced bradycardia which was not prevented by cutting the vagi or by atropine. The effect was evident with 0.05 mg. ATP – P and became stronger with larger doses, but on repeated injections the slowing became gradually less pronounced.

In *dogs* the injections of ATP into the left auricle or down to the base of the ascending aorta also caused pronounced slowing of the heart, which, like that seen in cats, was prevented by

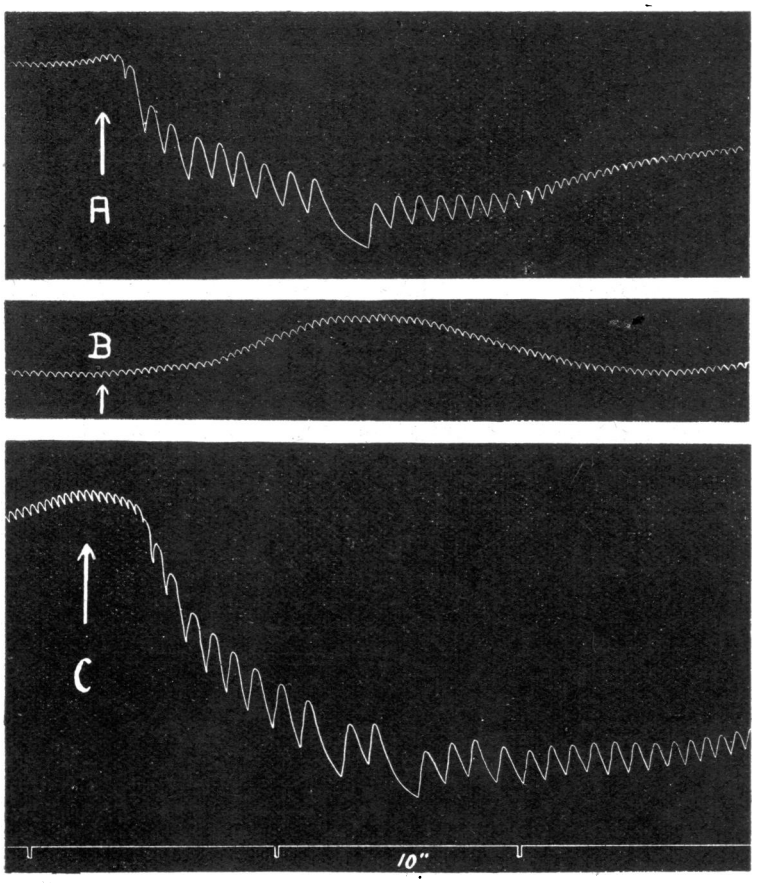


FIG. 4.—Carotid blood pressure of 3.2 kg. cat under chloralose; thorax open. Artificial respiration. At A and C 0.1 mg. ATP-P injected into left auricle, at C with carotid and vertebral arteries occluded. At B 0.1 mg. ATP-P injected into left vertebral artery. Time in 10 seconds.

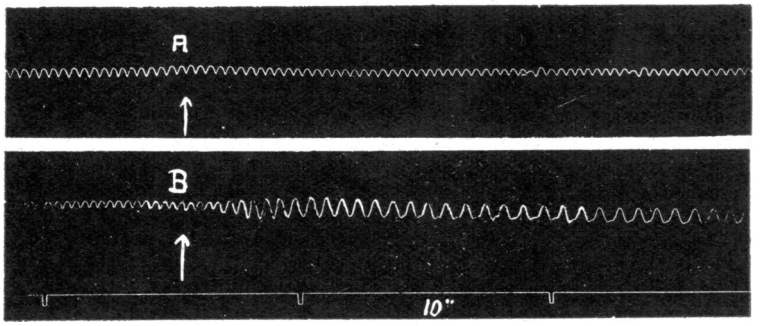


FIG. 5.—Blood pressure from femoral artery of 2 kg. cat under chloralose. Thorax open. Artificial ventilation. Heparin. Cannula tied into left coronary artery through subclavian artery. At A injection of 0.5 c.c. saline, at B of 0.1 mg. ATP-P into coronary artery. Art. blood pressure about 60 mm. Hg. Time in 10 seconds.

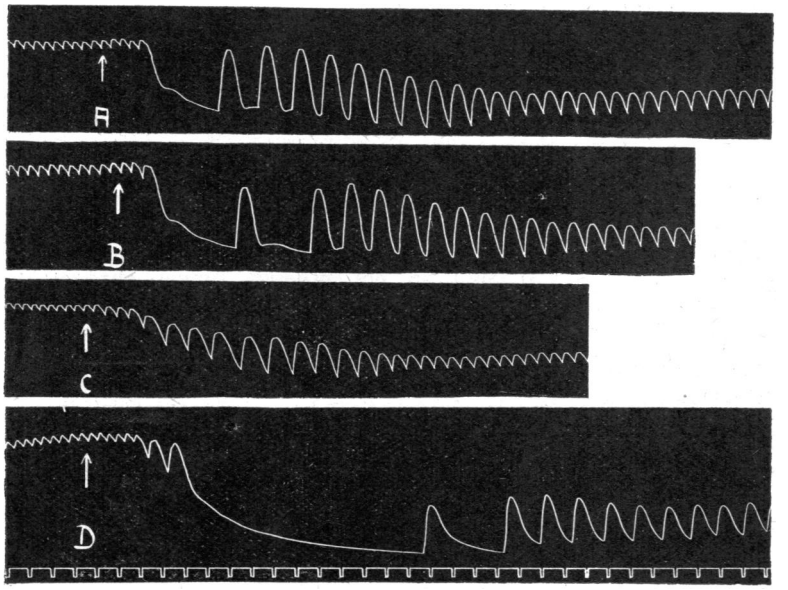


FIG. 6.—Carotid blood pressure of 11 kg. dog under chloralose. Thorax open; artificial ventilation. At A and B injections of 0.2 mg. ATP–P into ascending aorta at its base and at C and D into right vertebral artery. At B carotid and vertebral arteries occluded. Time in seconds.

cutting the vagi or by atropine. The effect was partly reflex, partly central in origin. In the experiment of Fig. 6, for instance, 0.2 mg. ATP – P injected low down into the ascending aorta produced pronounced bradycardia whether the carotid and vertebral arteries were left open (at A) or clamped (at B) before and during the injection. The effect therefore cannot be wholly due to stimulation of the cardio-inhibitor centre by ATP. The participation of a central factor, however, was shown by the bradycardia obtained when the same dose of ATP was injected into a vertebral artery; the slowing of the heart was weaker at C but stronger at D than that produced by injections into the left auricle at A and B. These doses of ATP had certainly no direct cardiac depressant action since they were ineffective after cutting the vagi. In another experiment central cardiac inhibition was obtained by injecting 0.2 mg. ATP – P into the carotid through a cannula in the lingual artery whilst the carotid was clamped at its aortic end. Ligating and cutting the sinus nerve did not abolish or diminish the effect, but if, subsequently, the vessels from the carotid leading to the brain were tied ATP became ineffective. The reverse procedure was adopted in the experiment shown in Fig. 7; the bradycardia became nearly abolished after the blood vessels to the brain had been tied, although the sinus nerve was kept intact. This was done between A and B. The slight slowing seen at B is probably not due to ATP, since saline injections into the carotid artery with its lower end occluded produced the same effect. The injections probably increased the pressure in the carotid artery for a short period and consequently stimulated the pressor receptors.

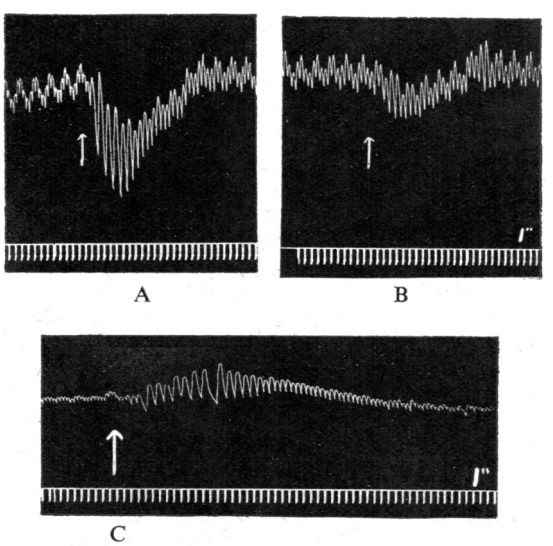


FIG. 7.—Arterial blood pressure of 8 kg. dog under chloralose. Injections of 0.2 mg. ATP–P into right carotid artery through cannula in lingual artery (at A and B) and into left coronary (at C). Between A and B mass ligation of right occipital, internal carotid and other small arteries leading to brain, but leaving sinus nerve intact. Between B and C thorax opened; artificial ventilation; heparin; cannula tied into left coronary artery for perfusion. Time in seconds.

Inhibition of the sympathetic is not involved in the reflex slowing of the heart, since the removal of the stellate ganglia did not diminish the effect.

The mechanism of the reflex bradycardia appears to be similar to that in cats and originates, at least to some extent, from the heart itself. In a few experiments the left coronary artery was perfused and an injection of ATP into it produced bradycardia provided the vagi were not cut (Fig. 7 C).

Respiratory changes in cats

An intravenous injection of 0.1 to 0.4 mg. ATP – P produces profound changes in pulmonary ventilation. There is often immediate cessation of movements lasting for 10 to 50 seconds. The apnoea precedes the fall of arterial blood pressure or occurs simultaneously. In some experiments apnoea was absent ; instead there was a period of shallow frequent respiratory movements. Both changes might be the sole effects or they might be followed by strong hyperventilation with increased depth and frequency of breathing and sometimes incomplete expiration. Some of these changes are illustrated in Fig. 8: at A there is a period of apnoea, at D a period of shallow frequent breathing in cats under chloralose ; at B and C there are periods of apnoea followed by strong hyperventilation, which at C occurs with incomplete expiration ; these tracings are from decerebrate cats. Injections of creatine phosphate (0.6 mg. CrP – P), adenylic acid (10 mg.) or sodium pyrophosphate (10 mg.) do not produce similar changes in pulmonary ventilation.

A complete analysis of the respiratory changes has not been carried out. The results so far obtained indicate, however, that several mechanisms are involved. ATP has a direct action on the respiratory centre and, in addition, seems to affect the centre indirectly by reflexes originating in the lungs, the impulses being carried via the vagi. No experiments have been performed to find out if it affects the centre also via the chemoreceptors.

The initial period of apnoea is brought about, partly at least, by a reflex via the vagus ; partly it

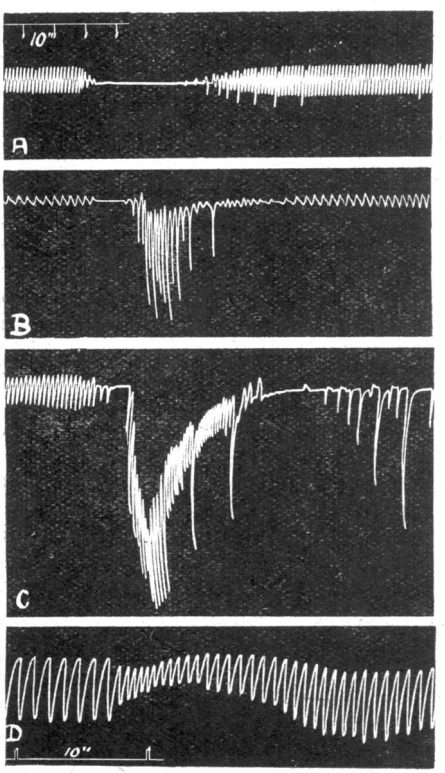


FIG. 8.—Record of respiration from four cats. A and D under chloralose, B and C decerebrate. Effect of intravenous injections of 0.1 mg. (in A and C) and 0.2 mg. (in B and D) of ATP–P. Time in 10 seconds shown on top of the figure for A, B and C and at the bottom for D.

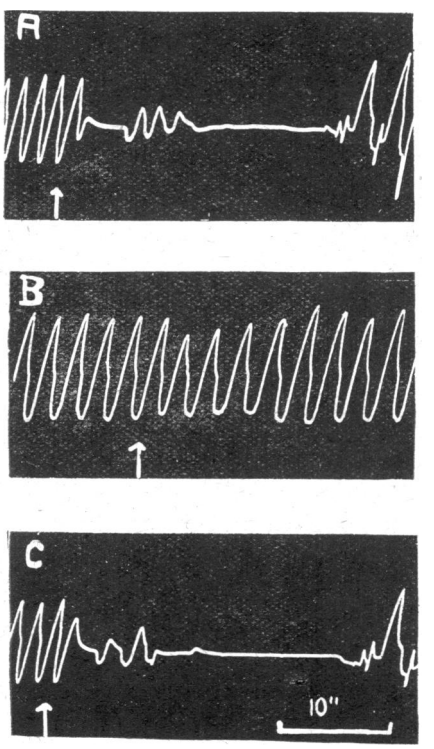


FIG. 9.—Record of respiration from 3 kg. cat under chloralose. At A, B and C intravenous injection of 0.4 mg. ATP–P . At B both vagi cooled at the neck. Time in 10 seconds.

may result from a central action of ATP. Cutting the vagi certainly reduces and sometimes even abolishes the apnoea. A certain caution, however, is necessary in the interpretation of this result, since repeated injections of ATP may cause reduced effects on respiration even without cutting the vagi. This argument does not apply to those experiments in which the vagi were cooled and the effect was found to be reversible. A striking experiment of this kind is illustrated in Fig. 9.

Some evidence in favour of the conception that the apnoea, as far as it is brought about reflexly, is due to stimulation of afferent fibres in the lungs, is given by an experiment in which the effect on respiration of 0.2 mg. ATP – P injected intravenously and into the ascending aorta has been compared. Instead of the initial apnoea produced by the intravenous injection there is a period of irregular breathing on arterial injection and, after cutting the vagi, the intravenous injection also produced these changes only.

A direct effect of ATP on the respiration centre is assumed from the fact that cutting the vagi usually did not wholly eliminate the respiratory changes and from the effects obtained when ATP was injected into a vertebral or carotid artery with denervated carotid sinus region. These injections produced respiratory changes, but they were weaker than those obtained with the same dose of ATP injected intravenously and with intact vagi.

Muscular contractions in cats

As mentioned earlier the experiments on circulation and respiration with ATP were the outcome of an incidental observation: i.e., widespread muscular contractions. These were obtained regularly in decerebrate cats on intravenous injection of 0.2 to 0.4 mg. ATP – P, but in cats under chloralose anaesthesia larger amounts of ATP had to be injected in order to produce this effect and even then it was not obtained regularly or in as pronounced a form as in decerebrate cats; only the latter therefore were used for further analysis.

The contractions consisted of a typical pattern. After a latency of between 15 to 20 seconds the forelegs became maximally extended and extreme opisthotonos developed. Sometimes the hind legs too became strongly extended. In several experiments, particularly when the cat was lying on its side with the head elevated, co-ordinated running movements occurred especially in the forelegs. Micturition, defaecation and strong peristalsis sometimes occurred. A similar pattern of muscular contractions to that observed after ATP could be elicited in the decerebrate cat on occluding the vertebral arteries with arterial clips; this was followed within less than 10 seconds by rigid extension of the forelegs and strong opisthotonos. It appeared possible therefore that anaemia of the central nervous system, caused by the fall in arterial blood pressure or stoppage of respiration, was responsible for the ATP contractions. But the effect occurred also when the depressor action of ATP was greatly reduced by injecting the ATP into the left ventricle, after atropine and with artificial ventilation. Central anaemia may, however, be a contributory factor when ATP is injected intravenously. When injected into the left ventricle the muscular contractions start after a shorter latency than after intravenous injections. This observation excludes also a reflex action of ATP originating in the lungs as the cause for the muscular effects. They occur also when the vagi nerves have been cut. In two out of five cats, however, the dose of ATP necessary to produce the muscular contractions on intravenous injection had to be doubled after the vagi were cut; thus afferent impulses in the vagi may perhaps participate to some extent in the initiation of the muscular contractions. Essentially, however, they are the result of an action of ATP on the central nervous system and therefore easily obtained when ATP is injected into a vertebral artery; when this is done the latency is a few seconds only. In decerebrate cats an action of ATP on supraspinal levels is probably the main cause of the muscular contractions, but ATP has a stimulating action also on the cervical cord. When the spinal cord was transected just above or below the origin of the first cervical nerve roots the injection of 0.2 to 0.3 mg. ATP – P into a vertebral artery produced muscular contractions but of a pattern different from that seen in the decerebrate cat. The main effect was on the hind legs and resembled the pattern of a typical scratch reflex on one or both legs. Rapidly alternating flexion and extension occurred at the ankle, the knee and less at the hip. In fact some flexion at the hip was more or less maintained during these rhythmic movements. The effect usually started on the side where the ATP was injected and if weak the movements occurred only at the ankle or at the ankle and knee. When the effect was pronounced the feet of both hind legs were brought to the shoulder region and exerted here the typical scratching movements. The effect on the forelegs was less pronounced and consisted of fine clonic movements and extension. A similar pattern of muscular contractions was produced in these cats on clamping one or both vertebral arteries. The contractions produced in these cats, even those on the hind limbs, resulted from an action of ATP on the cervical cord, because no effect or slight muscu-

lar contractions only were observed when the same dose of ATP was injected into the descending aorta or into the central end of the superior mesenteric artery with the iliac arteries clamped during the injections. With larger doses (over 0.4 mg. ATP—P), these injections caused muscular contractions but after a latency of more than 30 seconds, i.e., after the ATP had passed the lower body circulation, the legs and the left heart. The strong congestion of the pulmonary artery and the right heart preceding these contractions was good evidence that the ATP had reached the pulmonary circulation in effective concentration.

Discussion

The symptomatology of ATP when injected intravenously into cats is a complex one including peripheral, reflex and central mechanisms.

The profound and steep fall in arterial blood pressure is accounted for to a great extent by obstruction in the pulmonary circulation leading to a diminution in cardiac output. Gaddum and Holtz (1933) have described the constrictor effect of ATP on the pulmonary arteries, but its decisive role for the depressor action of this substance has not been recognized. According to these authors other phosphate compounds show the effect to a smaller degree, and in other animals, at least in the dog, the pulmonary vessels are less sensitive to ATP than in the cat. It would be of interest to know if in the dog the smooth muscles of the hepatic veins show instead a special sensitivity to ATP. A second direct effect contributing to the depressor action of ATP in cats is the vasodilatation it produces in the systemic circulation. There is also the possibility, mentioned before but not yet examined, of vasodilatation brought about by a reflex and central action of ATP and inhibiting the sympathetic tone. A third factor which will accentuate the depressor action of ATP is a bradycardia sufficiently strong to cause reduced cardiac output. The bradycardia is to some extent only accounted for by a central action of ATP, the explanation given by McDowall and by Bielchowsky *et al.* because it is abolished by cutting the vagi. This procedure, however, does not exclude the possibility of a reflex action of ATP. We could in fact show that ATP stimulates afferent fibres of the vagus in the heart and thus reflexly produces bradycardia. The effect resembles that produced by veratrin alkaloids (Dawes, 1947). In cats under chloralose this reflex mechanism accounts for the whole effect, but in decerebrate cats in which the centre appears to be particularly sensitive to ATP and in dogs under chloralose anaesthesia central actions of ATP contribute to the bradycardia. According to McDowall and to Bielschowsky *et al.* ATP has in cats also a direct depressant effect on cardiac muscle, which is not abolished after section of the vagi. These authors, however, have not excluded the possibility that changes resembling those produced by a direct cardiac depression may easily be simulated by obstruction in the pulmonary circulation with its consequent engorgement of the right and insufficient blood supply to the left heart. In those experiments in which we injected ATP into the left heart and thereby avoided pulmonary obstruction no signs of depression of the heart muscle were seen. The position is different in rabbits in which depression of the cardiac muscle occurs with ATP and has been demonstrated in isolated perfused hearts.

The profound changes in respiration produced by ATP have, so far as we know, not been described previously. A more detailed analysis than we have performed would be necessary in order to evaluate the different mechanisms involved in this effect. As far, however, as the results go, they show that ATP affects the respiratory centre directly as well as reflexly, via impulses in the vagi probably originating in the lungs.

The muscular contractions observed on injections of ATP intravenously or into the left heart are central effects of ATP. They may be accentuated by the circulatory and respiratory changes causing central anaemia, because a similar pattern of muscular movements could be obtained on occluding the vertebral arteries. They may be further accentuated by afferent impulses in the vagi, because in two cats at least the dose of ATP had to be increased after vagotomy in order to elicit the muscular contractions. This might be explained, however, simply by the fact that the vagal slowing of the heart is one of the circulatory events which leads to the central anaemia.

Buchthal, Engback, Sten-Knudsen, and Thomasen (1947) were the first to describe centrally produced muscle contractions when ATP was injected into a vertebral artery of a chloralosed cat. They recorded action potentials from the muscles of the forelegs and attributed the effect to a stimulation of the anterior horn cells in the cervical cord, since in their experiments the vertebral arteries were occluded at the level of the atlas and injections of Indian ink had shown that only the cervical segments were stained. We feel unable in our experiments to localize or to confine the stimulating action of ATP to these cells. The pattern of muscular movements varied in decerebrate and in spinal cats, but was the same whether produced by ATP or by occlusion of the vertebral artery. In

decerebrate cats a postural pattern was obtained similar to that seen when the head in these animals is strongly dorsiflexed: i.e., extensor spasm of the forelegs and opisthotonos. Such a pattern of muscular contractions suggests the possibility of a stimulating action of ATP on supraspinal levels. In spinal cats with the section just below the medulla oblongata or even at its lower end the pattern of muscular movements produced by ATP as well as by occlusion of the vertebral arteries resembled the scratch reflex. If the blood supply of the vertebral artery in these animals were limited to the cervical cord, this would indicate a representation of this reflex pattern in the cervical cord. It is interesting to note in this connection that injections of ATP into the descending aorta produced no or scarcely any contractions of the skeletal muscles, suggesting a special susceptibility of the cervical region of the cord to ATP.

The fall in arterial blood pressure, the reflex slowing of the heart, the obstruction in the pulmonary circulation, the vasodilatation, the peristalsis, micturition, vomiting, defaecation, and the muscular contractions of spinal and supraspinal origin, all this complex symptomatology is one which cannot be reproduced by other phosphate compounds, although they may have one or another action in common with ATP. On the other hand this symptomatology is not confined to ATP. For instance a striking similarity is found, at least in cats, with the effects produced by serum or plasma. The toxic actions of serum in cats were first examined by Brodie (1900). His records of the changes occurring in blood pressure and respiration are undistinguishable from the tracings we obtained with ATP. In cats, serum or plasma also produce a pronounced bradycardia, which was shown by Brodie to be of reflex origin, the reflex according to his analysis being initiated in the lungs. A re-examination of the effect, however, has shown that the heart is to some extent at least the site from which the reflex originates (Dawes and Feldberg, 1948). Like ATP, serum produces in cats strong contraction of the pulmonary vessels and on its intravenous injection this action may lead to the disappearance of the heart oscillations on the arterial blood pressure tracing and is in fact considered to be the main cause of the fall in pressure (Reid and Bick, 1942). Gilding and Nutt (1944) have further observed, on injection of stored plasma into cats, peristalsis, micturition, vomiting, defaecation, and muscular contractions of a pattern similar to that seen with ATP. They obtained the contractions only in decerebrate and not in chloralosed cats, but we have seen that ATP also was much more effective in this respect in a decerebrate cat. According to Gilding and Nutt the contractions are abolished by cutting the vagi and would therefore be of reflex origin. The contractions produced by ATP are of central origin; however, in some experiments section of the vagi necessitated a doubling of the dose of ATP in order to elicit the effect, and if the dose had not been increased the impression might easily have been gained of a reflex nature of the muscular contractions. The experiments with serum thus need re-examination in the light of these findings.

One might be tempted from such a close similarity of action to assume that serum and stored plasma owe their action to the presence of ATP; this, however, is not so; otherwise the "toxicity" of serum and plasma would not be confined to cats because ATP exerts its actions also in other animals. Adenylic compounds have been considered as the cause of the toxic action of serum or plasma. According to Zipf (1930), adenylic acid is identical with the "Frühgift" of serum, but adenylic acid cannot produce the toxic effects in cats. All the evidence available suggests in fact that the principle which makes serum and plasma so toxic for cats is a non-dialysable substance and probably a protein of the albumin class (Brodie, 1900; Reid and Bick, 1942; Gilding and Nutt, 1944). There is no evidence to suggest that ATP is linked to a protein constituent in serum and that in this linkage it would be active in cats but not in other animals.

Summary

1. In cats under chloralose or in decerebrate cats the intravenous injection of 0.2 to 0.4 mg. ATP – P causes (*a*) a steep fall in arterial blood pressure due to constriction of the pulmonary vessels, bradycardia, and vasodilatation, and (*b*) profound changes in respiration. In decerebrate cats these injections, in addition, regularly produce muscular contractions and often peristalsis, defaecation, vomiting, and micturition. In cats under chloralose anaesthesia ATP produces these additional effects only when given in larger doses and even then not regularly. The complex symptomatology of ATP cannot, or can in parts only, be reproduced by other phosphates.

2. The effect of ATP on the pulmonary vessels may be so strong that only little blood can enter the left heart. There is consequently a great reduction in cardiac output, which is to a great extent responsible for the steep fall in arterial blood pressure and which may cause obliteration of the heart oscillations on the blood pressure tracings.

3. The strong bradycardia produced by ATP, which causes a further reduction in cardiac output, results mainly from stimulation of afferent vagus fibres in the heart. This reflex mechanism accounts wholly for the bradycardia obtained in cats under chloralose. In decerebrate cats as well as in dogs under chloralose a central action of ATP contributes to the bradycardia seen after intravenous injections of ATP. On the other hand in rabbits the bradycardia produced by ATP is a peripheral effect.

4. The changes in respiration produced by ATP in cats consist of an initial period of cessation of respiration or of shallow frequent respiration often followed by a period of hyperventilation. ATP affects the respiratory centre directly and indirectly through impulses carried via the vagi and probably originating in the lungs.

5. The muscular contractions obtained in decerebrate cats on intravenous injections of ATP are of central origin and therefore also obtained on injection into a vertebral artery. They may be accentuated, however, by afferent impulses in the vagi and by central anaemia as a result of the circulatory and respiratory effects. The muscular contractions consist in the main of extensor spasm of the forelegs and episthotonos. In spinal cats with the cervical cord intact, intravenous or intravertebral injections of ATP cause muscular contractions, particularly in the hind limbs, of a pattern resembling the scratch reflex.

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