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SOME PHARMACOLOGICAL ACTIONS OF PALUDRINE

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Since the discovery of paludrine by Curd, Davey, and Rose (1945), few workers have studied its general pharmacological properties, though its low toxicity in comparison with many antimalarials is well known. Spinks, Tottey, and Maegraith (1946) found that the absorption, distribution, and excretion of paludrine in the rat resembled that of mepacrine. Spinks (1947) investigated the fate of paludrine given to rats and mice by mouth and found that it was rapidly absorbed from the stomach; a low recovery from the faeces and urine suggested that it was broken down in the body. Butler, Davey, and Spinks (1947) showed that the acute toxicity of paludrine varied both with the species and the method of administration. The intravenous or intraperitoneal injection of paludrine into rats and mice was followed by delayed deaths, the animals dying up to 24 hours after the injection; this was the more surprising in view of the fact that a closely related substance, 4430, did not exhibit this property. At first it was thought that these delayed deaths were caused by unusually prolonged retention of paludrine in the blood, but this was later disproved by measuring blood concentrations. Chicks given paludrine intravenously died within 15 min. or not at all, and from this and other evidence it was concluded that the metabolism of paludrine in chicks (and probably man) was different from that in rats and mice.

Hughes and Schmidt (1947) and Hughes, Schmidt, and Smith (1947a, b) also studied the absorption, toxicity, and excretion of paludrine in various animals. In the dog, paludrine induced copious salivation, loss of appetite, extreme cachexia, and cardiac arrhythmia. Feeding tests were performed in which paludrine was mixed with the food: the dogs refused to eat, and died from starvation. Chen and Anderson (1947) investigated the toxicity and then described in more detail the effects of paludrine in the body. In the anaesthetized or pithed cat, paludrine caused a temporary fall of the blood pressure. The respiratory rate was increased, but the depth diminished. Isolated loops of rabbit intestine were relaxed by paludrine and the isolated uterus of the guinea-pig was stimulated. Contractions of isolated intestine of the guinea-pig, induced by histamine, were inhibited. Innes (1947) showed that intramuscular injections of paludrine lactate caused necrosis, haemorrhage, oedema, and inflammatory exudate, with local involvement of nerves and vessels; this was most intense about 12 days after the injection. It is now generally believed that some metabolic product of paludrine is responsible for its antimalarial activity (Hawking and Perry, 1948), but so far attempts to identify this product have failed.

Experimental Results

Toxicity.—The acute intravenous toxicity of paludrine in mice of weights between 18 g. and 25 g. was studied. The LD50 was found to be 22 mg./kg., which agrees well with the results of other workers. As Butler, Davey, and Spinks (1947) observed, the toxicity of paludrine was sometimes delayed, and the figure for the LD50 given above was calculated after observing the mice for 72 hours after the injection. A possible explanation of the delayed toxicity was that paludrine interfered with some esterase mechanism in the animal. Blaschko, Chou, and Wajda (1947) showed that paludrine had an affinity for pseudocholinesterase and for the benzoylcholinesterase of guinea-pig's liver.

It was thought that the simultaneous injection of prostigmine, a very strong inhibitor of cholinesterase, might significantly alter the toxicity of paludrine. The intravenous toxicity of prostigmine in mice was therefore determined, and from the dose/response curve two doses of prostigmine were chosen; one which killed no mice (90 μg./kg.) and one which killed about 30 per cent of the mice (180 μg./kg.). Each of these doses was then injected simultaneously with the LD50 dose of paludrine (22 mg./kg.). The results are summarized in Table I.

TABLE I

INTRAVENOUS TOXICITY OF PALUDRINE, PROSTIGMINE, AND MIXTURES OF THE TWO

Group	Dose of drug	No. of mice used	Instant deaths	Delayed deaths			Total deaths	% Mortality
				0–24 hr.	24–48 hr.	48–72 hr.		
A	Prostigmine 90 μg./kg. ..	15	0	0	0	0	0	0
B	Prostigmine 180 μg./kg. ..	30	8	0	0	0	8	26.6
C	Paludrine 22 mg./kg.	30	0	8	4	4	16	53.4
D	Paludrine 22 mg./kg. + Prostigmine 90 μg./kg.	30	8	5	5	3	21	70
E	Paludrine 22 mg./kg. + Prostigmine 180 μg./kg.	30	29	0	0	0	29	96.7

Table I shows that the injection of prostigmine simultaneously with paludrine increased the immediate toxic effect. The immediate deaths, taken as a percentage of the total deaths, were increased from 0 per cent to 38 per cent by the addition of a non-toxic dose of prostigmine (90 μg./kg.), and to 100 per cent by a dose of prostigmine (180 μg./kg.), which alone killed about 27 per cent of animals.

Effect of paludrine on the cardiovascular system

Action on blood pressure and vessels.—In the anaesthetized animal an intravenous injection of paludrine usually caused a transient fall in blood pressure. In the perfusion of the dog's hind leg with heparinized blood, paludrine was shown to have a vasodilator action ; 2 mg. injected into the perfusion cannula decreased the pressure and increased the outflow of blood. This vasodilatation was diminished by neoantergan. The same effect was observed on the blood pressure of a cat anaesthetized with chloralose. Both paludrine (4 mg.) and histamine (10 μg.) caused a fall in blood pressure: this fall was reduced by the injection of benadryl. Thus the antihistamine agents, neoantergan and benadryl, reduced the vasodilatation caused by paludrine.

Paludrine antagonized the action of adrenaline on both the dog's perfused hind leg and the cat's blood pressure. The latter effect is shown in Fig. 1. In both preparations the vasoconstriction produced by adrenaline was reduced by paludrine.

Action on heart muscle.—The refractory period of the auricle was lengthened by paludrine. The compound was compared with quinidine by Dawes's method on the electrically stimulated rabbit auricles (Dawes, 1946), and it was found to be approximately one-eighth as active as quinidine (Table II).

On the isolated perfused cat heart (Langendorff preparation), paludrine (10–500 μg.) inhibited the amplitude and rate of beat according to the dose.

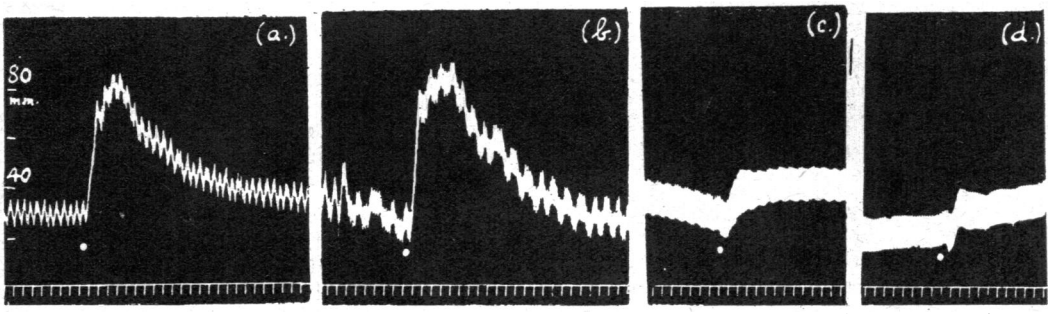

FIG. 1.—Blood pressure of chloralose cat from carotid artery. (*a*) Adrenaline (5 μg.); (*b*) adrenaline (5 μg.). Between (*b*) and (*c*) paludrine (80 mg.) was infused intravenously. (*c*) Adrenaline (5 μg.); (*d*) adrenaline (10 μg.). Time 10 sec.

TABLE II

PERCENTAGE LENGTHENING OF THE REFRACTORY PERIOD OF ISOLATED RABBIT AURICLES

The figures are averages of those from five experiments.

Paludrine		Quinidine	
Dose, mg.	% Increase of refractory period	Dose, mg.	% Increase of refractory period
1	4	0.25	9
2	9	0.5	16
4	14	1.0	23

The inhibition was accompanied by coronary dilatation.

Paludrine depressed both the contractility and the rate of beat of isolated rabbit auricles. At the same time the normal inhibitory action of acetylcholine was changed to a stimulation. If paludrine was allowed to act for a longer time the auricles stopped beating: acetylcholine would then restart the contractions. This action is described in more detail by Burn and Vane (1949). The isolated frog heart was also depressed by paludrine.

Action on striated muscle

On the isolated rectus abdominis of the frog paludrine had three distinct actions. When contractions of the muscle were obtained with acetylcholine, paludrine in very low concentration (5.0×10^{-7}) was found to augment these contractions. In concentrations higher than 10^{-6}, paludrine inhibited the action of acetylcholine. Finally, in concentrations of over 10^{-4}, paludrine alone caused the muscle to contract; the response to the same concentration of paludrine increased with successive applications. Thus, paludrine had a biphasic action on contractions evoked by acetylcholine; in small concentrations it augmented the contractions; in higher concentrations it depressed them.

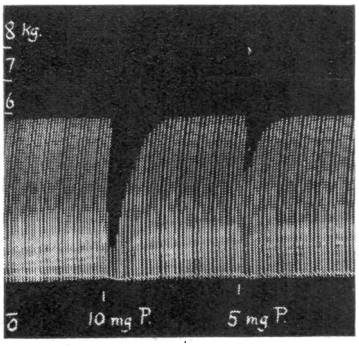

FIG. 2.—Cat sciatic-gastrocnemius preparation. Contractions of muscle evoked by maximal stimulation of the sciatic nerve by square waves at 24 shocks per min. Effect of two doses of paludrine injected into the artery.

Cat sciatic-gastrocnemius preparation. — The muscle twitch evoked by single maximal nerve volleys was depressed by paludrine (2–20 mg.) injected into the arterial blood stream (Fig. 2). This curariform action was also observed on the isolated phrenic nerve-diaphragm preparation of the rat.

Action on smooth muscle

Intestinal movements (in situ).—The intestinal movements of a cat, anaesthetized with chloralose, were recorded by a balloon tied into the duodenum. Alterations in volume of the balloon were recorded by a water manometer and piston recorder. A typical record is shown in Fig. 3; the upper tracing is the record of the intestinal movements; the lower one that of the blood pressure. The natural movements of the duodenum in this experiment were quite vigorous (*a*). An intravenous infusion of paludrine (1.3 mg./min.) lowered the tone of the muscle and abolished the natural movements. The paludrine infusion was stopped and the natural movements of the gut slowly returned; (*f*) shows the effect of a single injection of paludrine; the intestinal movements were stopped almost immediately. This effect was confirmed in three similar experiments. The blood pressure record in this experiment showed an interesting effect; the heart was irregular and often dropped beats (see *a* and *f*). The slow infusion of paludrine eliminated most of these, and after the single injection of paludrine all the irregularities disappeared.

Intestinal movements after vagal stimulation.—In some cats prepared for the above experiment the natural movements of the gut were small. In these animals the right vagus nerve was exposed in the neck, cut, and the peripheral end stimulated by an induction coil for 30 sec.; this produced a burst of motility in the duodenum. An intravenous infusion of paludrine decreased the response of the intestine to vagal stimulation; the activity recovered when the infusion was stopped.

Isolated intestine.—The contractions of isolated guinea-pig ileum elicited by acetylcholine were inhibited by paludrine, and so were the contractions elicited by histamine. The inhibitions of the contractions were of the same order in each case. Paludrine, in a concentration of 2×10^{-6}, decreased the natural tone of isolated rabbit duodenum; at

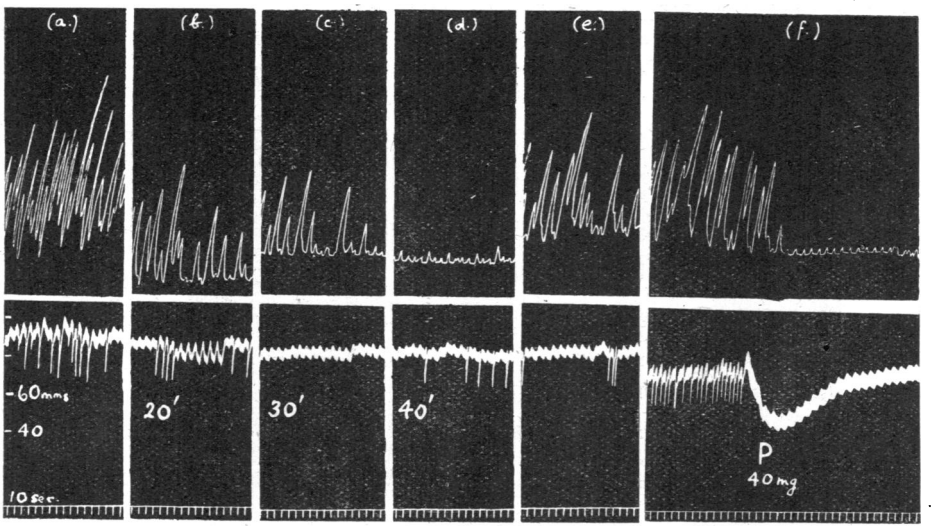

FIG. 3.—Chloralose cat. Top: intestinal movements, recorded by a balloon in the duodenum. Bottom: blood pressure from carotid artery. (*a*) Natural movements; infusion of paludrine started at 1.3 mg./min.; (*b*) 20 min. after start of infusion; (*c*) 30 min.; (*d*) 40 min. Infusion stopped (52 mg. infused). Activity began to return; (*e*) 10 min. later. Eventually normal activity returned, as shown in (*f*) (1 hour later). A single injection of paludrine (40 mg.) again stopped the movements. Note effect on blood pressure and dropped beats. Time 10 sec.

the same time it augmented the response of the muscle to adrenaline.

Splenic volume.—The volume of the spleen of the cat was recorded by means of a plethysmograph attached to a piston recorder. Paludrine injected intravenously had little effect on the blood pressure, but dilated the spleen. This was confirmed in six other experiments.

Action on bronchioles.—The bronchial tone was recorded by the method described by Konzett and Roessler (1940), and Emmelin, Kahlson, and Wicksell (1941), using the recorder described by Halpern (1942).

The guinea-pig was anaesthetized with urethane, and cannulae were inserted into the left jugular vein and the trachea. The lungs were artificially respired by a pump; the excess air was measured by the apparatus referred to above, so that increased excursion of the recorder on the smoked paper indicated broncho-constriction.

Injections of paludrine (1–20 mg.) into the jugular vein had in themselves no effect, but greatly enhanced the broncho-constriction caused by histamine. Fig. 4 shows this effect. Histamine (2 μg.) produced regular constrictions of the bronchioles: the injection of paludrine (10 mg.) increased the histamine effect (*a*). Paludrine (20 mg.), whilst having no effect by itself, increased the histamine constriction so much that the guinea-pig died (*b*).

Respiration

Rabbits anaesthetized with urethane were used. Respiration was recorded by Gaddum's method (1941). Paludrine (8–20 mg.) reduced the respiration, but the depression did not last as long as the fall in blood pressure which accompanied it.

In further experiments a section of the right vagus nerve was exposed in the neck. The nerve was cut and the central stump was stimulated by an induction coil for 5 sec. once every minute. This produced a constant inhibition of the respiration as shown in Fig. 5*a*. Paludrine (8 mg.) injected intravenously caused a transitory depression of the respiration, after which the vagal effect was abolished. The stimulation once every minute was continued and the vagal depression gradually returned to its normal value in about 20 min. In another experiment in which paludrine (4 mg.) was injected, the effect reached its maximum at about 10 min. after the injection. In both these rabbits, as in four other experiments which gave similar results, the blood pressure remained constant, except for a small transient fall on injection of the paludrine.

Ganglionic transmission

The superior cervical ganglion of the cat was perfused with Ringer-Locke solution by the method of Kibjakow (1933). Stimulation of the

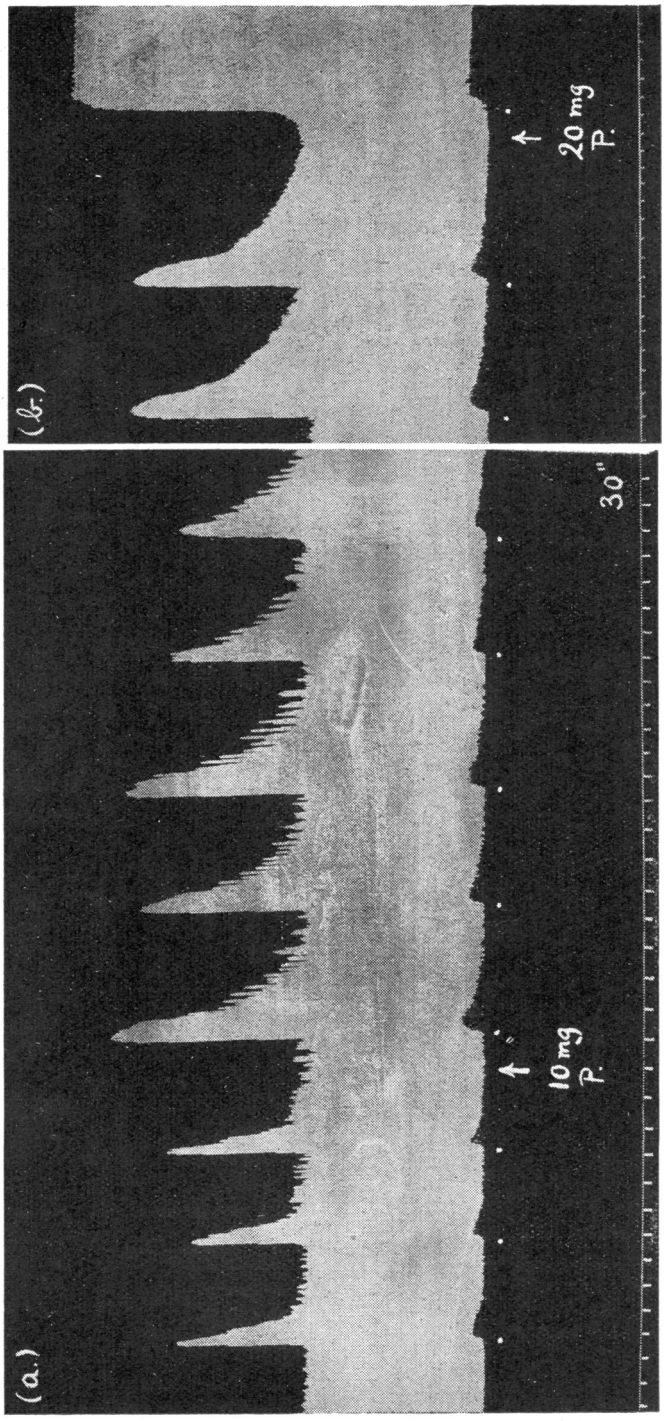

FIG. 4.—(*a*) Record of constrictions of guinea-pig bronchioles induced by histamine (2 μg.) intravenously injected at dot. At arrow paludrine (10 mg.) was injected. It had no action by itself, but potentiated the histamine response. (*b*) Constrictions caused by histamine (3 μg.). At arrow paludrine (20 mg.) was injected. This had no effect, but the next histamine injection killed the animal. Time 30 sec.

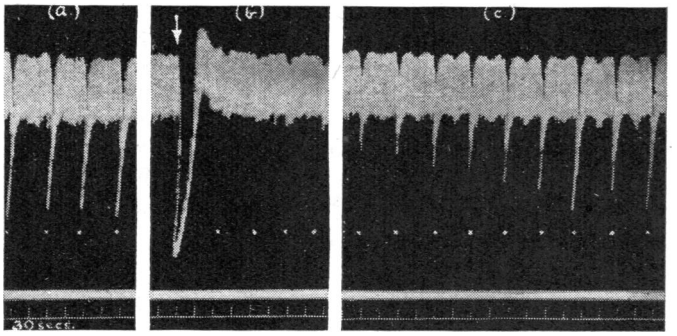

FIG. 5.—Respiration of rabbit recorded by Gaddum's method. (*a*) Central end of cut vagus nerve stimulated for 5 sec. once every minute. (*b*) Paludrine (8 mg.) injected at arrow. This caused transient depression of respiration. Effect of vagal stimulation abolished. (*c*) Effect of stimulation gradually returning, the first stimulation being 11 min. after injection of paludrine. Time 30 sec.

preganglionic fibres produced a contraction of the nictitating membrane: this was recorded on smoked paper by a lever with a frontal writing point. Injection of paludrine into the infusion cannula depressed the response of the nictitating membrane (Fig. 6); this appeared with doses of paludrine exceeding 200 μg.

Action on secretions

The action of paludrine on salivary secretion in cats was compared with that of atropine by the method of Bülbring and Dawes (1945). Although paludrine (4–16 mg.) gave a very transient inhibition, the inhibition was never as large as that produced by atropine (2 μg.).

The antidiuresis evoked by pituitary (posterior lobe) extract in rats was slightly prolonged by paludrine (10 mg./kg.) when injected with the pituitary extract; the method described by Burn (1931) was used.

Paludrine was found to inhibit the histamine-induced gastric secretion in cats, and also the gastric secretion evoked by a test meal in man. This effect is described in other papers (Burn and Vane, 1948; Vane, Walker, and Wynn Parry, 1948).

DISCUSSION

Late deaths after the intravenous injection of paludrine have been observed in rats and mice but not in chicks (Butler, Davey, and Spinks, 1947). It is interesting to note that late deaths also occurred after the oral administration of pamaquin, certuna, and mepacrine to fowls, and that the nervous symptoms which accompanied the delayed deaths prevented ingestion of food, the deaths sometimes being hastened by starvation (Köhlschütter, Zipf, and Triller, 1943). Paludrine given to rats, mice, dogs, and monkeys in sublethal doses also diminished the intake of food, as described by Hughes, Schmidt, and Smith (1947b). Thus, delayed deaths and loss of appetite seem to be common properties of the synthetic antimalarial drugs pamaquin, certuna, mepacrine, and paludrine.

Late deaths might also be due to interference with some enzyme system. Blaschko, Chou, and Wajda (1947) found that certain cholinesterases were inhibited by paludrine; the experimental results described in this paper show that when prostigmine was injected simultaneously with paludrine into mice the immediate deaths were increased and the delayed deaths decreased. This would be expected if the deaths were due to inhibition of the esterase by paludrine.

On the other hand, in most of its actions paludrine was found to antagonize the effect of vagal stimulation or of acetylcholine. The action of

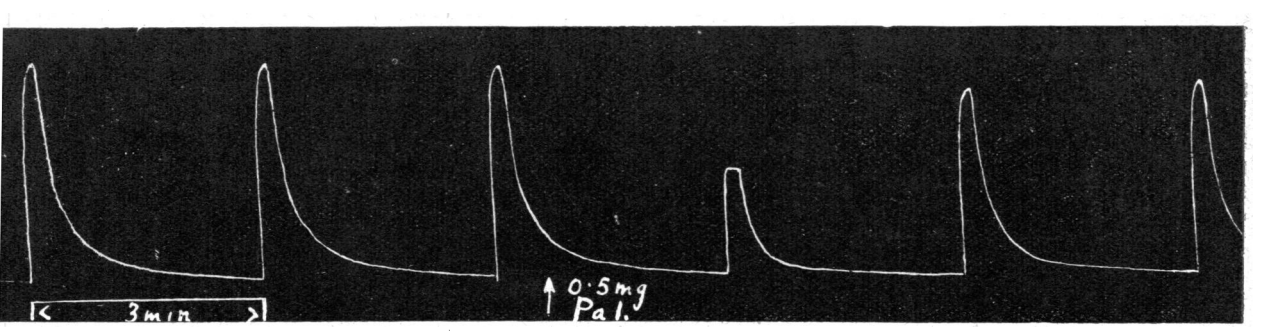

FIG. 6.—Perfusion of superior cervical ganglion. Stimulation of preganglionic fibres produced contractions of the nictitating membrane shown in tracing. Paludrine (0.5 mg.) injected into the perfusion fluid partially blocked the transmission.

acetylcholine was inhibited on the cat intestine *in situ*, on the isolated guinea-pig ileum, on the isolated rabbit auricles, and on the isolated frog rectus muscle. In smooth muscle the natural tonus was reduced. Quinine, quinidine, cinchonine, pamaquin, and mepacrine were found by Keogh and Shaw (1943, 1944) first to stimulate, and then relax, isolated rat's intestine. The same authors also showed that the depressor response of the cat's blood pressure to acetylcholine was reduced by quinine. Hiatt, Brown, Quinn, and Macduffie (1945) found that quinine, quinidine, and cinchonine all inhibited the action of the vagus on the heart.

Dawes (1946) discussed the relationship between the inhibitory action of drugs on the acetylcholine response and their effect in prolonging the refractory period of the heart. Paludrine has been found to lengthen the refractory period and to impair the contractility of isolated rabbit auricles. Here, too, the other antimalarial drugs have a similar effect. Quinine, mepacrine, and pamaquin decreased the heart rate and lengthened the conduction time as shown by the e.c.g. in man and in dogs (Molitor, 1941). The lengthening of the refractory period by quinidine, and to a lesser extent by quinine, is well known. Smith and Stoekle (1946) showed that mepacrine impaired the contractility of the heart.

A curariform action of paludrine has been demonstrated on the perfused superior cervical ganglion, on the cat sciatic-gastrocnemius preparation, and on the rat phrenic nerve-diaphragm preparation. Harvey (1939) described a curariform action of quinine on the neuromuscular junction.

Paludrine in large doses has been shown to antagonize the vasoconstriction caused by adrenaline in the dog's perfused hind leg and on the cat's blood pressure. Keogh and Shaw (1943, 1944) found that quinine in large doses reversed the action of adrenaline on the cat's blood pressure.

Molitor (1941) showed that mepacrine and pamaquin produced vasodilatation, as does quinine. In this work it has been shown that paludrine also produces vasodilatation in the dog's perfused hind leg and in the cat. The dilatation was partially abolished by the antihistamine agents, neoantergan and benadryl. This, with the work of MacIntosh and Paton (1947), who found that certain biguanides and amidines released histamine from muscle, suggested that paludrine may also release histamine from the tissues. The relationship between paludrine and histamine is very puzzling: whereas paludrine inhibited gastric secretion evoked by histamine and reduced the response of isolated guinea-pig ileum to histamine, it potentiated the action of histamine on the guinea-pig lungs. On the other hand the antihistamine agents, benadryl and neoantergan, which abolish the effect of histamine in most tissues but potentiate the gastric secretion caused by histamine (Wood, 1948), reduced the action of paludrine on the systemic vessels.

Summary

Certain pharmacological actions of paludrine are described.

1. In most experiments, paludrine antagonized the action of acetylcholine or of vagal stimulation: contractions of the isolated frog rectus muscle and guinea-pig ileum were inhibited by paludrine and the normal action of acetylcholine on isolated rabbit auricles was abolished. The effects of vagal stimulation on the cat intestine and rabbit respiration were also reduced, as was the natural tonus of the intestine.

2. As with other drugs which antagonize acetylcholine, paludrine lengthened the refractory period of auricular tissue. It also had a curariform action on the cat sciatic-gastrocnemius, the rat phrenic nerve-diaphragm, and the perfused superior cervical ganglion preparations.

3. Paludrine reduced or abolished the vasoconstrictor action of adrenaline in the dog's perfused hind leg and on the cat blood pressure.

4. Paludrine caused vasodilatation of the perfused dog hind leg and in the cat. This dilatation was reduced by injection of antihistamine agents, which suggested that paludrine might release histamine from the tissues. If this is so, then the relationship between histamine and paludrine is difficult to understand, for, whereas paludrine potentiates the constrictor effect of histamine on the guinea-pig lungs, it inhibits histamine-induced gastric secretion in cats. It also inhibits contractions of isolated guinea-pig ileum evoked by histamine.

5. The delayed toxicity observed in mice after the intravenous injection of paludrine was changed by the simultaneous injection of prostigmine, which increased the proportion of immediate deaths.

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