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PHARMACOLOGICAL ACTIONS OF PURE MUSCARINE CHLORIDE

BY

P. J. FRASER

From the Pharmacology Laboratory, The Wellcome Research Laboratories, Beckenham, Kent

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The action of chromatographically pure crystalline muscarine chloride, prepared from *Amanita muscaria*, has been compared with acetylcholine chloride (ACh) on a number of different organs from a variety of species. Muscarine caused spasm *in vivo* and *in vitro* of muscles of the gut, uterus, urinary bladder, and bronchus. It also caused contraction of the horse ureter and carotid artery chain *in vitro* and slowed the isolated auricles of the guinea-pig and rabbit, and the frog heart.

Muscarine caused a drop in blood pressure, although *in vitro* it produced either constriction or dilatation of the blood vessels of the rabbit ear.

All these actions resembled those of acetylcholine, though muscarine was usually more potent. Muscarine effects were readily prevented by atropine sulphate. It had a slight action on the frog rectus abdominis muscle, causing a contracture at high concentrations. Muscarine was destroyed neither by pepsin nor by boiling at any *p*H. It was inactive by mouth in a monkey in a quantity many times that which would cause poisoning by ingestion of *Amanita muscaria* in the human being. Muscarine neither inhibited nor was hydrolysed by either true- or pseudo-cholinesterase. Muscarine chloride did not cause paralysis of the neuromuscular junctions of the rat diaphragm or of the cat gastrocnemius.

Since Schmiedeberg and Koppe's paper in 1869, muscarine has been studied extensively. In the early literature, the term muscarine was applied not only to the active principle obtained from *Amanita muscaria,* but also to a compound derived from the action of nitric acid on choline which had a similar action. At one time these compounds were thought to be identical and the resulting confusion is well shown in Fühner's review on muscarine (1923).

A small quantity of crystalline, highly purified muscarine chloride was recently prepared from *Amanita muscaria* by Dr. S. Wilkinson. Its pharmacological actions have been compared with those of acetylcholine and are reported in the present paper.

METHODS

Experiments in vitro

All isolated tissues were bathed in appropriate oxygenated physiological saline solutions at 37° C., unless otherwise indicated.

Intestine.—Segments of ileum 3 to 5 cm. long were taken from the mouse, guinea-pig, and rabbit. Longitudinal strips (3 to 5 cm. by 3 to 5 mm.) from the dog, horse, and monkey were used. A circular strip from the horse was also tested. Similar lengths of rat colon and frog duodenum were also used. Frog intestine was examined in frog Ringer at room temperature.

Uterus.—Longitudinal strips from the mouse, guinea-pig, rabbit, rat, horse, and dog, and a circular strip from the horse were used. Mouse uteri were tested at 34° C.

Bladder.—Longitudinal strips of bladder from the guinea-pig, dog, rat, and monkey, and longitudinal and circular strips from the rabbit and horse were used. Strips from the frog bladder were tested at room temperature.

Tracheal Chain.—Chains, prepared from circular sections of the trachea of the guinea-pig and rabbit, were suspended in oxygenated van Dyke and Hastings solution at 37° C.

Arterial Chain.—These were made from circular sections of the horse carotid artery at 37° C.

Ureter.—Strips of ureter (4 to 5 cm.) from the horse were used.

Auricle.—The beating auricle from the guinea-pig or the rabbit was suspended in an oxygenated physiological saline at 30° C.

Heart.—The Clark (1912) frog heart preparation was set up at room temperature and perfused with frog Ringer.

Frog Rectus.—This was suspended in oxygenated frog Ringer at 5 to 10° C.

Leech.—The dorsal muscle of the leech was suspended in oxygenated Ringer at room temperature.

Ear.—The isolated ear of the rabbit was perfused at constant pressure with 0.9% NaCl solution at room temperature and the rate of venous outflow recorded.

Diaphragm.—The isolated rat phrenic nerve diaphragm preparation of Bülbring (1946) was immersed in oxygenated physiological saline solution at 37° C., and the nerve was stimulated by single rectangular pulses of 2 V. with a duration of 0.23 msec. at the rate of 5/min.

Experiments in vivo

Respiration.—Respiration was recorded from a cannula in the trachea of the rabbit or cat.

Blood Pressure.—The blood pressure was recorded manometrically from a cannula in the carotid artery of the rabbit, cat, or dog.

Uterus.—Movements of the non-pregnant uterus of the rabbit, cat, or dog were recorded by connecting the uterus *in situ* by a thread to a weighted lever, the peritoneal cavity being filled with saline at 37° C.

Bladder.—Changes in intravesical pressure in the cat or dog were recorded by tying a catheter through the bladder neck and connecting it to a wide bore saline reservoir and a tambour recorder.

Intestine.—Contractions of the longitudinal muscles of the intestines of the cat or dog were recorded by anchoring one end of a length of intestine to the abdominal wall and attaching the free end by a thread to a weighted lever. Contractions of the circular muscle were recorded by inserting a water-inflated balloon into a section of intestine and connecting it to a tambour recorder.

Gastrocnemius.—The sciatic nerve of the cat was stimulated maximally by single stimuli from a neon stimulator at 6 discharges/min.; contractions of the gastrocnemius were recorded by a thread from the muscle to a spring-loaded lever. Injections were made into the femoral vein.

Bronchoconstriction.—This was recorded in the guinea-pig or rabbit. Spontaneous respiration was abolished by an overdose of pentobarbitone sodium and the animal maintained on artificial respiration. Bronchoconstriction was recorded as a decrease in intrathoracic pressure.

In all *in vitro* and *in vivo* experiments the activities of muscarine chloride and acetylcholine chloride were compared by producing, whenever possible, equivalent responses.

Anaesthetics.—For the bronchoconstriction test in rabbits or guinea-pigs, ether and pentobarbitone sodium were used. Otherwise, urethane was used for rabbits, and pentobarbitone sodium for cats and dogs.

Conscious Animal.—A monkey (female 6.7 kg.) was given muscarine by stomach tube and by intraperitoneal injection.

Toxicity.—Groups of 5 albino mice were used, injections being made into a tail vein.

Manometric Methods.—A Warburg respirometer with single side arm flasks was used. The substrate—acetyl-β-methylcholine chloride for true cholinesterase and benzoylcholine chloride for pseudo-cholinesterase—was placed in the side arm. The enzyme preparation—human red blood cells for true cholinesterase and human plasma for pseudo-cholinesterase—was placed in the base of the flask with muscarine chloride, and Ringer-bicarbonate solution was added, bringing the total volume to 0.3 ml. Final concentrations were: acetyl-β-methylcholine, 0.03 M; benzoylcholine, 0.015 M; and muscarine chloride, 5.0×10^{-4} M. The whole system was gassed for 10 min. with a 95% N_2 and 5% CO_2 mixture. The muscarine and the enzyme were left in contact at 37° C. for 30 min. before tipping in the substrate. The rate of evolution of CO_2 was then compared with that of a control in the absence of muscarine.

Stability.—Muscarine chloride at concentrations of 1.0, 10.0, and 100.0 μg./ml. in 0.9% NaCl solution was stored in air at 0° C. for 18 months and its activity on the rabbit ileum was compared with a freshly prepared sample. Muscarine was boiled at *p*H 1.0, 6.0, and 11.0 for 10 min. and similarly tested. It was also tested after digestion in pepsin (10.0 mg./ml.) at *p*H 3.0 at 37° C. for 18 hr., and also after incubation in human oxalated blood at 37° C. for 1 hr.

Results

Stability.—Muscarine chloride showed no loss of potency when stored in solution for 18 months at 0° C. It was stable to boiling for 10 min. at *p*H 1.0, 6.0, and 11.0; resistant to peptic digestion at *p*H 3.0 at 37° C. for 18 hr., and showed no loss of activity when incubated in human oxalated blood at 37° C. for 1 hr.

Toxicity.—The mouse i.v. LD50 for muscarine chloride was calculated to be 0.23 mg./kg. and for acetylcholine chloride 33.05 mg./kg., thus muscarine was 143 times more toxic than acetylcholine. Signs of poisoning were similar, acetylcholine being lethal almost immediately and muscarine after 3 to 10 min. Death never occurred later than 10 min. after the injection.

Experiments on Isolated Organs

Intestines.—Both muscarine and acetylcholine caused spasm of the isolated gut from the eight species tested. Sensitivity varied considerably between species (Table I) and between specimens from the same species. Atropine sulphate always blocked the responses to both compounds. Muscarine spasm usually developed more slowly than that caused by acetylcholine and took longer to recover after washing the preparation. Except on the mouse ileum and the rat colon, muscarine was

TABLE I

COMPARISON OF SPASMOGENIC ACTIVITIES OF MUSCARINE AND ACETYLCHOLINE ON ISOLATED INTESTINAL MUSCLES OF VARIOUS SPECIES

Species	Preparation	Equiactive Concentrations (mμg./ml.)		Activity Ratio of Muscarine to ACh (ACh=1)	
		ACh	Muscarine		Mean
Mouse ..	Ileum	33·0	40·0	0·83	0·83
		330·0	400·0	0·83	
Guinea-pig	,,	7·5	3·0	2·5	
		10·0	4·0	2·5	4·1
		15·0	10·0	1·5	
		200·0	20·0	10·0	
Rabbit ..	,,	10·0	10·0	1·0	
		40·0	30·0	1·3	2·6
		100·0	33·0	3·0	
		100·0	20·0	5·0	
Dog ..	,,	33·0	8·0	4·1	
	(longitudinal)	33·0	8·0	4·1	4·1
Rat ..	Colon	166·0	155·0	1·0	
		33·0	66·0	0·5	0·75
Frog ..	Duodenum	3,300·0	100·0	33·0	
		1,650·0	50·0	33·0	33·0
Horse ..	Ileum	16·6	12·5	1·3	
	(longitudinal)	330·0	10·0	33·0	
		330·0	10·0	33·0	22·5
,, ..	Ileum	330·0	6·0	55·0	
	(circular)	1,000·0	6·0	167·0	111·0
Monkey ..	Ileum	100·0	10·0	10·0	
	(longitudinal)	60·0	6·0	10·0	10·0

TABLE II

COMPARISON OF SPASMOGENIC ACTIVITIES OF MUSCARINE AND ACETYLCHOLINE ON ISOLATED UTERINE MUSCLES OF VARIOUS SPECIES

Species	Preparation	Equiactive Concentrations (mμg./ml.)		Activity Ratio of Muscarine to ACh (ACh=1)	
		ACh	Muscarine		Mean
Mouse ..	Longitudinal	1,000·0	1,000·0	1·0	1·0
		166·0	150·0	1·1	
		1,660·0	1,660·0	1·0	
Guinea-pig	,,	666·0	100·0	6·7	
		333·0	83·0	4·0	5·3
Rabbit* ..	,,	250·0	666·0	0·37	
		166·0	333·0	0·5	0·43
Rat ..	,,	500·0	300·0	1·67	
		267·0	234·0	1·14	
		333·0	84·0	4·0	2·27
Horse ..	,,	67·0	10·0	6·7	
		166·0	25·0	6·7	
		1,000·0	100·0	10·0	
		1,670·0	167·0	10·0	8·35
,, ..	Circular	100·0	12·0	8·5	
		500·0	50·0	10·0	
		1,000·0	100·0	10·0	
		670·0	67·0	10·0	
		330·0	33·0	10·0	9·7
Dog ..	Longitudinal	1,000·0	100·0	10·0	

* Muscarine abolished ACh response temporarily.

more active than acetylcholine in producing spasm; again, except on these two tissues, physostigmine increased the sensitivity of the muscle to acetylcholine, and the ratio of activities of the two compounds became unity. In the two exceptional preparations, physostigmine did not always cause an increase in the sensitivity to acetylcholine.

Uterus.—Table II gives the results on the isolated uterus from six different species. As on the gut, the spasm caused by muscarine was slower in onset and recovery after washing was slower than with acetylcholine. Atropine prevented the action of each compound. Muscarine was more active than acetylcholine except on the uterus of the mouse and of the rabbit. An interesting finding with the rabbit uterus preparation was that, after recovery from a muscarine-induced spasm, the response to acetylcholine was temporarily abolished.

Bladder.—In all seven species tested muscarine was more active than acetylcholine (Table III). Muscarine responses were slower and were prevented by atropine.

TABLE III

COMPARISON OF SPASMOGENIC ACTIVITIES OF MUSCARINE AND ACETYLCHOLINE ON ISOLATED MUSCLES FROM THE URINARY BLADDER OF VARIOUS SPECIES

Species	Preparation	Equiactive Concentrations (mμg./ml.)		Activity Ratio of Muscarine to ACh (ACh=1)	
		ACh	Muscarine		Mean
Guinea-pig	Longitudinal	133·0	12·0	8·3	
		1,000·0	58·0	17·2	
		667·0	18·0	37·0	20·8
Rabbit ..	,,	130·0	4·0	32·5	
		160·0	8·0	20·0	
		333·0	12·0	27·8	
		100·0	6·0	16·6	
		333·0	12·0	27·8	24·9
,, ..	Circular	667·0	13·0	51·5	
Dog ..	Longitudinal	333·0	13·0	25·3	
		167·0	3·0	55·8	
		167·0	2·0	83·5	
		333·0	3·0	111·0	
		333·0	2·0	167·0	88·5
Rat ..	,,	67·0	17·0	3·9	
		200·0	23·0	8·7	6·3
Frog ..	,,	12·0	5·7	2·0	
		6·7	3·3	2·0	
		6·7	2·8	2·4	
		200·0	33·0	6·0	
		167·0	16·7	10·0	4·5
Horse ..	,,	33·0	3·3	10·0	
		66·0	6·6	10·0	10·0
,, ..	Circular	50·0	6·7	7·5	
		33·0	3·3	10·0	8·8
Monkey ..	Longitudinal	1,330·0	6·7	200·0	
		1,000·0	5·0	200·0	200·0

Other Isolated Organs.—Table IV summarizes the results obtained with other preparations.

With the tracheal chain preparation of the guinea-pig and of the rabbit, muscarine was many times more active than acetylcholine in causing spasm. Its action was slower with a latent period of about 2 to 3 min., an effect not seen with acetylcholine.

The difference in the rate of action was even more marked in the contraction of the carotid artery chain of the horse in which the spasmogenic action was not apparent until 5 min. after muscarine was added to the bath.

TABLE IV

COMPARISON OF SPASMOGENIC ACTIONS OF MUSCARINE AND ACETYLCHOLINE ON OTHER ISOLATED ORGANS FROM VARIOUS SPECIES

Species	Preparation	Equiactive Concentrations (mμg./ml.)		Activity Ratio of Muscarine to ACh (ACh=1)	
		ACh	Muscarine		Mean
Guinea-pig	Tracheal chain	3,000·0	50·0	60·0	
		1,670·0	6·7	250·0	155·0
Rabbit ..	,,	1,670·0	6·7	250·0	
Horse ..	Carotid artery chain	1,000·0	400·0	2·5	
		500·0	150·0	3·3	2·9
,, ..	Ureter	1,000·0	216·0	4·6	
		1,000·0	13·0	77·0	
		1,000·0	234·0	4·3	28·6
Guinea-pig	Auricle	16·7	16·7	1·0	
		33·0	40·0	0·83	
		25·0	16·7	1·5	1·1
Rabbit ..	,,	200·0	30·0	6·7	
		400·0	40·0	10·0	
		100·0	27·5	3·6	
		100·0	50·0	2·0	
		200·0	35·0	5·7	5·6
Frog ..	Heart	13·4	20·0	0·67	
,, ..	Rectus	50·0	10,000·0	0·005	

The ureter of the horse contracted well to both compounds and recovery of the preparation after washing out either compound was rapid.

Muscarine and acetylcholine were equiactive in producing slowing of the rate and decrease of the size of the beat of the isolated guinea-pig auricle; on the rabbit auricle, muscarine was 5.5 times more active than acetylcholine. Fibrillation of the rabbit auricle was converted by both drugs to a slow regular beating of the organ. Muscarine was 10 times more potent than acetylcholine and, after washing, the period before fibrillation recurred was 14 times longer.

On the isolated frog heart, both muscarine and acetylcholine caused a slowing of the rate and a decrease in the amplitude of the beat in both the auricle and the ventricle. Recovery was slow and never quite complete.

All the above effects were prevented by atropine. The action of atropine on the fibrillating auricle was not tested.

An unexpected finding was that muscarine in a concentration of 10,000 mμg./ml. caused a very slow contracture of the isolated frog rectus abdominis muscle equivalent in height to the much more rapid response caused by 50 mμg./ml. acetylcholine. The muscle was slow to recover after washing out the muscarine. The responses to both drugs were prevented by (+)-tubocurarine.

On the dorsal muscle of the leech, a response to acetylcholine could only be obtained after physostigmine. No response was obtained with muscarine in concentrations up to 1,000 mμg./ml. alone or after physostigmine.

In the isolated perfused rabbit ear, muscarine and acetylcholine were equiactive, both producing constriction or both producing dilatation of the blood vessels in any one ear.

Muscarine in concentrations up to 10,000 mμg./ml. had no action on the rat phrenic nerve diaphragm preparation *in vitro*, nor did it influence the paralysis caused by (+)-tubocurarine or suxamethonium.

Experiments in vivo

Respiration.—Both drugs caused temporary decrease of the respiratory movements in the rabbit or cat. Muscarine was four times more active than acetylcholine in both species.

Blood Pressure.—Again muscarine was about four times more potent than acetylcholine in causing a fall in blood pressure in the rabbit, cat, or dog. The response to muscarine was slower and more prolonged than that with acetylcholine.

Organs.—Muscarine caused contractions of the non-pregnant uterus of the rabbit, the cat, or the dog, the urinary bladder of the cat or dog, and both circular and longitudinal muscles of the gut of the cat or dog. Acetylcholine acted similarly to but was less active than muscarine. The organs of the dog were particularly sensitive to muscarine. This accounts for the much greater activity ratio of muscarine to acetylcholine in this species than in the other species studied (Table V).

TABLE V

COMPARISON OF VARIOUS ACTIONS OF MUSCARINE AND ACETYLCHOLINE *IN VIVO*

Action on	Species	Type of Response	Mean Activity Ratio of Muscarine to ACh (ACh = 1)	Minimum Active Dose of Muscarine i.v. (μg./kg.)
Intrathoracic pressure	Guinea-pig	Bronchoconstriction	5·0	2·0
	Rabbit	,,	4·5	2·0
Respiration ..	Rabbit	Depression	4·0	5·0
	Cat	,,	4·0	5·0
Blood pressure	Rabbit	Fall	4·0	5·0
	Cat	,,	5·0	1·0
	Dog	,,	Approx. 3·5	0·5
Uterus ..	Rabbit	Contraction	4·0	10·0
	Cat	,,	>4·0*	5·0
	Dog	,,	Approx. 250·0	0·5
Bladder ..	Cat	,,	20·0	0·5
	Dog	,,	Approx. 300·0	0·1
Gut (circ.) ..	Cat	,,	>4·0*	5·0
	Dog	,,	Approx. 40·0	0·5
Gut (long.) ..	Cat	,,	>10·0*	2·0
	Dog	,,	Approx. 500·0	0·2

* No response obtained with acetylcholine.

No response from the uterus and gut of the cat was obtained with acetylcholine up to 20 μg./kg. The cat bladder proved interesting in that the response to acetylcholine was greatly reduced after a dose of muscarine.

Atropine sulphate prevented all responses obtained with both drugs *in vivo*.

The cat gastrocnemius was not paralysed by muscarine or by acetylcholine.

In the guinea-pig and rabbit, bronchoconstriction, obtained with both muscarine and acetylcholine, could be prevented by atropine. Recovery from both drugs was spontaneous, but that from muscarine was slower than with acetylcholine.

The following experiment on a monkey was of interest as it showed that muscarine apparently had little activity by mouth. An intraperitoneal injection of 2 mg. of muscarine was rapidly followed by profuse mucous salivation and vomiting. The animal became prostrate with apparently severe abdominal discomfort. There was miosis, and respiration was deep and slow with uncoordinated abdominal and thoracic movements. Atropine completely abolished all these signs within 5 min. with the exception of the miosis, which gave place to mydriasis after about 40 min.

A fortnight later, this monkey was given 2 mg. muscarine by mouth and no signs of poisoning developed during the next 5 hr. Then, 0.5 mg. was given intraperitoneally and severe toxic effects occurred which were abolished by atropine.

Action on Cholinesterases.—At high concentrations, acetylcholine inhibits both true and pseudocholinesterase (Alles and Hawes, 1940). Muscarine at a concentration of 5.0×10^{-4} M had no inhibitory action on either true or pseudocholinesterase, nor was it hydrolysed by either enzyme.

DISCUSSION

The actions of pure muscarine chloride have been shown to conform to the type of actions of acetylcholine and other drugs known as "muscarinic," that is, "acting on smooth muscles and glandular cells that are innervated by postganglionic cholinergic nerve fibres." This study has been largely limited to a quantitative and qualitative comparison of the actions of muscarine chloride and acetylcholine chloride on smooth muscle systems.

Both *in vitro* and *in vivo*, muscarine caused spasm of the gut, uterus, and urinary bladder of a number of different species. Muscarine was not hydrolysed by cholinesterases. It was usually more active than acetylcholine w/w, and its action was always slower and more prolonged. It caused constriction of the bronchi of the guinea-pig and rabbit and spasm of the horse ureter and carotid artery *in vitro*. This last observation was of interest as muscarine is generally considered to be a vasodilator. However, in the isolated rabbit ear, muscarine caused either constriction or dilatation of the blood vessels, but, *in vivo* in the cat and dog, it caused a marked drop in blood pressure; it slowed the isolated frog heart and the isolated auricles of the guinea-pig and rabbit. All these actions were shared with acetylcholine and were readily prevented by atropine sulphate. In most preparations tested, treatment with physostigmine increased the sensitivity of the muscle to acetylcholine, and its activity then became comparable to that of muscarine although it remained faster in action. Physostigmine never altered the sensitivity of the isolated muscle to muscarine as has been claimed by Waser (1955) to occur *in vivo*.

At the neuromuscular junction it has not been possible to demonstrate any action of muscarine in experiments on the rat diaphragm, on the cat gastrocnemius preparation, or on the dorsal muscle of the leech, thus confirming the results of

Ambache, Perry and Robertson (1956) and of Zaimis (quoted by these authors). On the other hand the frog rectus abdominis differed from the other muscles in that 10,000 mμg./ml. of muscarine produced a slow contracture. Ambache, Perry, and Robertson (personal communication) did not employ such high concentrations of muscarine.

Although muscarine was stable to boiling and to pepsin, no action by mouth was found in the monkey despite the fact that the amount given was many times that which would cause poisoning by the ingestion of *Amanita muscaria* in the human being.

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