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The effect of cholinesterase inhibitors on the antimuscarinic effect of hemicholinium-3 (HC-3) in the rat

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The effect of hemicholinium-3 (HC-3) on responses of the rat isolated bladder and ileum to acetylcholine and carbachol was investigated in the absence and presence of a number of anticholinesterases. Responses of the bladder to acetylcholine were potentiated by DFP, edrophonium, BW284C51 and physostigmine but were unaffected by the specific butyrylcholinesterase inhibitor iso-OMPA. Responses to carbachol were not potentiated by the anticholinesterases. HC-3 ($1{\cdot}7 \times 10^{-4}$ M) inhibited responses to carbachol without affecting those to acetylcholine. In the presence of physostigmine or DFP responses to acetylcholine were inhibited by HC-3 but no such inhibition was observed in the presence of BW284C51, edrophonium or iso-OMPA or a combination of the latter two anticholinesterases. Responses to carbachol were also inhibited to a greater extent in the presence of DFP. In the ileum, responses to acetylcholine were increased in the presence of DFP, edrophonium and physostigmine but were unaffected by iso-OMPA. Responses to carbachol were not increased by any of the anticholinesterases. HC-3 ($2{\cdot}8 \times 10^{-4}$ M) inhibited responses to both acetylcholine and carbachol in the ileum and the degree of inhibition was not significantly altered by the presence of any of the anticholinesterases used.

Although a weak anticholinesterase, HC-3 was also found to decrease the inhibitory action of physostigmine on the hydrolysis of acetylcholine by homogenates of rat ileum. A similar effect was noted with DFP but not with edrophonium.

The results obtained do not support a prejunctional action for HC-3 in antagonizing responses to carbachol. It is concluded that in addition to an inhibitory action on the postjunctional muscarinic receptor HC-3 may interfere with the anticholinesterase activity of some cholinesterase inhibitors such as physostigmine and DFP but not edrophonium.

Hemicholinium-3 (HC-3) is widely known to be an inhibitor of the choline uptake process in cholinergic nerves. On a wide variety of isolated tissues from the guinea-pig, Bertolini, Greggia & Ferrari (1967) found that HC-3 had antimuscarinic activity. However, an anomalous situation exists in that there are some tissues in which HC-3 is ineffective against acetylcholine alone but will antagonize other muscarinic agonists such as carbachol, oxotremorine and tetramethylammonium (TMA). These tissues include guinea-pig taenia coli (Mitchelson, 1971), rat bladder and rabbit ileum (György, Pfeifer & Kenyeres, 1970). To explain this anomaly György & others (1970) suggested that HC-3 has only a prejunctional site of action in the rat bladder. Thus, carbachol and oxotremorine, but not acetylcholine, would act prejunctionally to release endogenous transmitter and this effect is inhibited by HC-3. Further, they found that responses to acetylcholine in the presence of physostigmine were inhibited by HC-3 and they suggested that this was because acetylcholine, in the presence of the anticholinesterase, could also release endogenous transmitter.

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The aim of this investigation was to determine whether HC-3 was acting prejunctionally or was modifying postjunctional muscarinic receptors as suggested by Mitchelson (1971) on the taenia coli. The investigation involved the use of a number of anticholinesterases to determine how they modified the action of HC-3 on responses to acetylcholine or carbachol in the rat bladder and ileum and how HC-3 modified the activity of the anticholinesterases.

METHODS

Isolated tissue experiments

All experiments were with male Wistar rats (Glaxo strain).

Rat bladder. The rat bladder was set up in a 10 ml organ bath according to Huković, Rand & Vanov (1965). Electrical stimulation of parasympathetic nerves to the bladder was with supramaximal voltage and 2 ms pulse duration at frequencies of 5–80 Hz for 5 s with at least 3 min intervals between successive stimulations. Responses to agonists were recorded on a Grass polygraph at 5 min intervals, the agonists remaining in contact with the tissue until it had fully responded to the drugs (60–120 s).

Rat ileum. A length of non-terminal ileum, 2–3 cm, was set up in a 10 ml organ bath containing McEwen solution (1956) gassed with 5% carbon dioxide in oxygen and maintained at 37 ± 0·05°. Contractions were recorded under a resting tension of 1 g via an Ether strain gauge, type EF1, on a Gilson M5P polygraph. Agonists were added every 3–4 min and remained in contact with the tissue for 30 s. The tissues were washed twice between addition of agonists.

In isolated tissue experiments involving the use of DFP, the anticholinesterase was incubated with the tissue for either 30 min (bladder) or 10 min (ileum). The tissue was then washed repeatedly over a further 5–10 min to remove excess DFP and the responses to agonists were then re-determined without further addition of the anticholinesterase. A similar procedure was followed with iso-OMPA except that the period of incubation was 10 min. All other anticholinesterases and HC-3 were incubated with the tissue for at least 15 min before re-determining responses to agonists and were replaced in the bath medium immediately after each washing of the tissue. To ensure that equilibrium with the anticholinesterase or HC-3 had been reached all responses were determined at least in duplicate until constant responses were obtained.

Dose-ratio determinations. With ileum, a full log dose-response curve was obtained using at least 4 concentrations of the agonist. With the bladder at least 3 concentrations of the agonist were used to obtain responses lying on the linear portion of the log dose-response curve. For each tissue a line of best fit was plotted through those points lying between 15 and 85% of the maximum response and the shift in the log dose-response curve produced by anticholinesterases or antagonists was measured at the ED50 concentration. Although some anticholinesterases produced changes in the slope of the dose-response curve to acetylcholine in the bladder, measurement at the ED50 provided some measure of the degree of potentiation.

Cholinesterase determinations. The ability of a homogenate of rat ileum to hydrolyse acetylcholine in the presence of combinations of cholinesterase inhibitors and HC-3 was measured by a pH-stat titrimetric method. Lengths of ileum were homogenized in liquid nitrogen and suspended in 0·9% saline. The final reaction mixture of 15 ml contained 50 to 100 mg tissue.

The rate of hydrolysis of acetylcholine was determined at pH 7·4 at 37° by titrating the liberated acetic acid with 0·02 M NaOH in an atmosphere of carbon dioxide-free nitrogen. After an initial incubation period of 5–10 min during which the spontaneous production of acid by the homogenate was measured acetylcholine (10^{-2} M) was added and the rate of hydrolysis was measured for 2–5 min. The reaction rate was corrected for the spontaneous production of acid by the homogenate.

In experiments involving the use of DFP the tissue was incubated at 37° in the presence of the inhibitor for exactly 30 min before addition of substrate. For edrophonium and physostigmine the inhibitor was added to the tissue 10–15 min before addition of the substrate. These incubation times corresponded to those used in the isolated tissue experiments.

Drugs used were: acetylcholine chloride (Sigma), butyrylcholine iodide (Sigma), BW284C51 (1,5-bis(4-allyldimethylammonium phenyl)pentane-3-one dibromide) (Wellcome), carbachol (Koch-Light), diisopropylphosphorofluoridate (DFP) (Sigma), edrophonium chloride (Roche), hemicholinium-3 dibromide (Aldrich), iso-OMPA (Koch-Light), physostigmine sulphate (Macfarlan Smith).

RESULTS

Effect of anticholinesterases in bladder

DFP. DFP ($5·4 \times 10^{-6}$ M) potentiated responses to acetylcholine but slightly inhibited those to carbachol (Table 1). Responses to electrical stimulation of the bladder were also increased markedly in duration and magnitude.

Edrophonium. At $4·1 \times 10^{-5}$M, edrophonium increased responses to acetylcholine and nerve stimulation but slightly inhibited responses to carbachol (Table 1). A ten-fold increase in the concentration of edrophonium produced greater potentiation of the responses to acetylcholine and also greater inhibition of responses to carbachol (Table 1).

Iso-OMPA. Although responses to butyrylcholine were potentiated 3·5-fold by iso-OMPA ($2·9 \times$

Table 1. *The effect of anticholinesterases on responses to acetylcholine (ACh) and carbachol (CCh) in the rat isolated bladder.*

Anticholinesterase	Concn. (M)	Dose-ratio obtained at ED50* ACh	CCh
DFP	$5·4 \times 10^{-6}$	+45·5±10·2 (6)	−2·0±0·4 (3)
Edrophonium	$4·1 \times 10^{-5}$	+12·4±2·5 (4)	−1·8±0·2 (7)
	$4·1 \times 10^{-4}$	+28·8±8·5 (4)	−4·4±0·6 (4)
Iso-OMPA	$2·9 \times 10^{-4}$	0 (3)	0 (4)
Physostigmine	$3·1 \times 10^{-6}$	+208·9±88·9 (3)	0 (5)

* Dose-ratios are shown as the mean ± s.e.m. (no. of experiments), + indicates potentiation of the response to the agonist by the anticholinesterase; − indicates inhibition.

10^{-4} M), responses to acetylcholine and nerve stimulation were unaffected (Table 1).

Physostigmine. At 0·6 — 3·1 × 10^{-6} M physotigmine produced a marked leftward shift of the dose-response line for acetylcholine (Table 1) and increased responses to nerve stimulation. Responses to carbachol were not affected. There was no increase in bladder tone but spontaneous activity increased slightly.

BW284C51. In two experiments, BW284C51 (1·8 × 10^{-5} M) potentiated responses to acetylcholine 4·6- and 4·8-fold, respectively.

Effect of anticholinesterases in ileum

DFP. Responses to acetylcholine were potentiated by DFP but the degree of potentiation was much less than in bladder, being only 3-fold despite the use of a 10-fold higher concentration of DFP (Table 2). Still higher concentrations of DFP induced spontaneous contractions.

Table 2. *The effect of anticholinesterases on responses to acetylcholine (ACh) and carbachol (CCh) in the rat isolated ileum.*

		Dose-ratio obtained at ED50*	
Anticholinesterase	Concn (M)	ACh	CCh
DFP	5·4 × 10^{-5}	+3·1±0·3 (3)	0 (6)
Edrophonium	4·1 × 10^{-5}	+2·0±0·2 (11)	0 (6)
iso-OMPA	2·9 × 10^{-5}	0 (9)	0 (6)
Physostigmine	3·1 × 10^{-8}	+2·1±0·4 (4)	0 (4)

* See Table 1.

Edrophonium. At 4·1 × 10^{-5} M, the drug caused only a small potentiation of responses to acetylcholine (Table 2) A 10-fold higher concentration produced no further change.

Iso-OMPA. Despite a slight potentiation (1·4-fold) of responses to butyrylcholine by iso-OMPA (2·9 × 10^{-5} M) responses to acetylcholine were not affected (Table 2). Higher concentrations of iso-OMPA inhibited the responses.

Physostigmine. At 3·1 × 10^{-8} M, physostigmine caused a small potentiation of responses to acetylcholine (Table 2). Above this concentration marked spontaneous activity developed.

Responses to carbachol in the ileum were not affected by any of the anticholinesterases.

Effect of HC-3 on responses to agonists

Bladder. HC-3 (1·7 × 10^{-4} M) produced a 2·3-fold shift of the dose-response curve to carbachol without affecting the response to acetylcholine (Table 3) or nerve stimulation. Twice the dose of HC-3 did not affect responses to acetylcholine. After incubation of the tissue with DFP responses to carbachol were inhibited by HC-3 to a significantly greater extent ($P < 0.05$, 2-tailed t-test). Responses to acetylcholine were also inhibited by HC-3 in the presence of DFP.

Edrophonium (4·1 × 10^{-5} M) did not alter the effect of HC-3 on responses to acetylcholine or carbachol. However, at 4·1 × 10^{-4} M it significantly lowered ($P < 0.001$, 2-tailed t-test) the HC-3 induced inhibition of responses to carbachol (Table 3).

Table 3. *The effect of hemicholinium-3 (HC-3) (1·7 × 10^{-4} M) in rat isolated bladder on responses to acetylcholine (ACh) and carbachol (CCh) in the absence and presence of anticholinesterases.*

		Dose-ratio obtained with HC-3*				P†
Anticholin-esterase	Concn (10^{-6} M)	ACh		CCh		
None	—	0	(5)	−2·3±0·1	(5)	—
DFP	5·4	−1·7±0·2	(6)	−2·8±0·1	(4)	<0·05
Edro-phonium	41	0	(4)	−2·3±0·3	(6)	N.S.
	410	0	(4)	−1·3±0·1	(4)	<0·001
iso-OMPA	290	+1·6±0·2	(3)	−2·6±0·3	(4)	N.S.
Edro-phonium + iso-OMPA	410 290	0	(3)	−1·5±0·1	(3)	<0·01
Physo-stigmine	3·1	−2·4±0·1	(4)	−2·5±0·2	(5)	N.S.

* See Table 1.
† Probability of the significance of the difference between values obtained for carbachol in the absence and presence of a cholinesterase inhibitor; N.S. = not significant.

Iso-OMPA did not affect the action of HC-3 on responses to carbachol but caused HC-3 to slightly potentiate (1·6-fold) responses to acetylcholine (Table 3). A combination of edrophonium (4·1 × 10^{-4} M) and iso-OMPA (2·9 × 10^{-4} M) led to the same results as edrophonium (4·1 × 10^{-4} M) alone.

Responses to acetylcholine were not affected by HC-3 in the presence of BW284C51. However, in the presence of physostigmine, HC-3 antagonized responses to acetylcholine (Table 3). The inhibitory effect of HC-3 on responses to carbachol was unaltered in the presence of physostigmine.

Ileum. HC-3 (0·7 — 2·8 × 10^{-4} M) inhibited responses to both acetylcholine and carbachol. The dose-ratio for acetylcholine produced by 0·7 × 10^{-4}M HC-3 was 2·9 ± 0·3 (n = 5) (mean ± s.e.m.) and for carbachol was 4·5 ± 0·4 (n = 4). The values obtained at 2·8 × 10^{-4} M HC-3 are n = 5·4 ± 0·5 (12) for acetylcholine and n = 10·4 ± 2·5 (9) for carbachol. The mean dose ratios for carbachol are greater than those for acetylcholine with the higher concentration of HC-3, the difference being signifi-

cant ($P < 0.05$, 2-tailed t-test). However, the use of any anticholinesterase at the concentrations shown in Table 2 did not significantly alter the effectiveness of HC-3 as an inhibitor of either acetylcholine or carbachol ($P > 0.05$, 2-tailed t-test).

Anticholinesterase experiments

In preliminary experiments with homogenates of bladder or ileum, HC-3 decreased the effectiveness of physostigmine as an inhibitor of the hydrolysis of acetylcholine. Because ileum experiments required fewer animals, further work was with homogenates of ileum. In contrast to its action in decreasing the effectiveness of physostigmine as an inhibitor (Table 4), HC-3 ($1.7–3.5 \times 10^{-4}$ M) had no significant effect ($P < 0.05$) on the inhibition of hydrolysis produced by edrophonium (4.1×10^{-4} M).

Table 4. *The effect of hemicholinium-3 (HC-3) and physostigmine, alone and in combination, on the rate of hydrolysis of acetylcholine* (10^{-2} M) *by homogenates of rat ileum.*

Concn of HC-3 (10^{-4} M)	Rate of hydrolysis (% of untreated ileum)†		
	Concn of physostigmine (10^{-7} M)		
	0	2	4
0	100	28·9±2·3 (6)	18·4±1·0 (9)
1·7	96·5±2·0 (11)	41·3±2·2 (6)*	30·0±1·9 (9)*
3·5	81·3±3·1 (8)	45·6±2·4 (3)*	33·1 ±4·3(7)**

* Values marked with an asterisk are significantly different ($P < 0.001$ 2-tailed t test) from the corresponding value in the absence of HC-3. ** $0.01 > P > 0.001$.
† Values shown are mean ± s.e.m. (number of observations).

DFP ($0.5 - 5 \times 10^{-9}$ M) produced less inhibition of the hydrolysis of acetylcholine by rat ileum if HC-3 (1.7×10^{-4}M) was added immediately before the 30 min incubation period with DFP (Table 5). In contrast, when HC-3 was added to the incubation medium for the last 2 min of the 30 min period there was no such reduction in the effectiveness of DFP (5×10^{-9} M) (Table 5).

Table 5. *The effect of HC-3* (1.7×10^{-4}M) *and DFP in combination on the rate of hydrolysis of acetylcholine* (10^{-2}M) *by homogenates of rat ileum.*

Concn of HC-3 (10^{-4} M)	Rate of hydrolysis (% of untreated ileum) at DFP concn: ($\times 10^{-9}$ M)			
0	0	0·5	1	5
1·7 (added before DFP)	100 93·8 ±4·0 (12)	51·3 ±7·1 (12) 60·9 ±7·6 (12)*	34·6 ±9·1 (9) 46·7 ±11·1(9)*	4·7 ±1·3 (3) 9·7 ±0·7 (3)*
1·7 (added after DFP)	0 100 109·5 ±7·6 (4)	47·5 ±4·2 (4) 44·4 ±5·8 (4) N.S.		

N.S. = Not significantly different from, corresponding value obtained in absence of HC-3.
* Significantly different from corresponding value obtained in the absence of hemicholinium (2-tailed paired t test, $P < 0.05$).

DISCUSSION

Anticholinesterases produce a number of actions on cholinergic neuroeffector junctions apart from effects arising from inhibition of cholinesterases. For example, it has been suggested that edrophonium and physostigmine increase release of acetylcholine from cholinergic nerve endings (Blaber & Bowman, 1959; Cox, Hecker & Weston, 1970) and that physostigmine can directly excite muscarinic receptors in guinea-pig ileum (Cox & Lomas, 1972). Edrophonium, BW284C51 and iso-OMPA have been reported to inhibit muscarinic receptors in various tissues in the rat (Ambache & Lessin, 1955; Roberts & Konjovic, 1969; Jamieson, 1963). Such actions may complicate interpretation of results. Therefore, concentrations of anticholinesterases were used which did not produce any increase in tone, an effect suggestive of acetylcholine release by the anticholinesterase. Also any depression of responses to carbachol was noted since this was suggestive of an inhibitory action on postjunctional receptors on the smooth muscle.

In rat tissues, physostigmine (Todrick, 1954) and DFP (Ord & Thompson, 1950) inhibit both acetylcholinesterase and butyrylcholinesterase and the two inhibitors potentiated responses to nervous stimulation as well as to acetylcholine in the rat bladder. Similar findings were made with edrophonium and BW284C51 which are selective inhibitors of acetylcholinesterase in several species (Austin & Berry, 1953; Bayliss & Todrick, 1956; Mitchelson, 1971). In contrast, iso-OMPA a selective inhibitor of butyrylcholinesterase in the rat (Aldridge, 1953; Bayliss & Todrick, 1956) failed to increase responses to nerve stimulation or acetylcholine although potentiating responses to butyrylcholine. These results suggest that only acetylcholinesterase is involved in the hydrolysis of both endogenous and exogenous acetylcholine when concentrations applicable to the isolated bladder experiments are investigated.

In the bladder no potentiation by anticholinesterases of responses to carbachol was observed (Table 1); the responses were, in fact, inhibited in the presence of DFP and edrophonium. Thus, there is no evidence here that carbachol can act presynaptically to release endogenous transmitter

because a response dependent on such a release should be potentiated by anticholinesterases. That potentiation of an endogenous release of acetylcholine by anticholinesterases could be detected was demonstrated by the anticholinesterases increasing the responses to nerve stimulation in the bladder.

The results with HC-3 and bladder (Table 3) confirm those reported by György, Pfeifer & Kenyeres (1970). HC-3 antagonized responses to carbachol but was ineffective against acetylcholine unless physostigmine was present. In the presence of DFP, HC-3 also produced some inhibition of responses to acetylcholine but was also significantly more effective as an inhibitor of responses to carbachol. In contrast, the use of the three specific inhibitors, edrophonium, BW284C51 or iso-OMPA did not reveal any inhibitory action of HC-3 on acetylcholine responses.

In the ileum, responses to acetylcholine were also increased in the presence of DFP, physostigmine or edrophonium, but not iso-OMPA (Table 2). However, the degree of potentiation was small compared to the results on bladder. Responses to carbachol in the ileum were not potentiated by any of the anticholinesterases. Jamieson (1963) concluded that only acetylcholinesterase was important in the hydrolysis of endogenous acetylcholine in rat ileum so if carbachol was acting by stimulating intramural ganglia or by releasing acetylcholine from postganglionic cholinergic nerve endings, some potentiation of responses following treatment with inhibitors of acetylcholinesterase would have been anticipated.

In the ileum, HC-3 was a more effective inhibitor of cholinomimetics than in the bladder but responses to carbachol were still inhibited to a greater extent than those to acetylcholine. Treatment of the ileum with anticholinesterases did not produce any significantly different results from those in untreated ileum. This may be because the use of higher concentrations of DFP and physostigmine was precluded by increased spontaneous activity.

György & others (1970) suggested that HC-3 acted presynaptically to block responses to carbachol and oxotremorine and only inhibited responses to acetylcholine when added anticholinesterases allowed it to act presynaptically. Our results are compatible with a postjunctional inhibition of the muscarinic receptor. In the taenia coli of the guinea-pig, HC-3 antagonizes responses to TMA and carbachol without affecting responses to acetylcholine (Mitchelson, 1971). In this tissue as well, no evidence could be found for a prejunctional action of TMA in producing contraction of the tissue.

This inhibitory effect of HC-3 against muscarinic agonists is immediate and therefore not associated with any decline in the available stores of cholinergic transmitter due to a block of the choline transport mechanism. Such a decline occurs slowly since exhaustion of existing transmitter stores must first occur (Rand & Ridelhalgh, 1965). Huković, & others (1965) found that HC-3 only reduced responses to nervous stimulation after about 3 h of repeated stimulation whereas in our experiments no inhibition of nerve transmission was present when responses to carbachol were inhibited by HC-3.

Recent evidence suggests that HC-3 acts allosterically as a metaffinoid antagonist of the muscarinic receptor in the guinea-pig atrium (Madden & Mitchelson, 1975). In this tissue, HC-3 antagonizes acetylcholine and carbachol but the latter is antagonized to the greater extent. HC-3 could also be having the same action on rat bladder. As the inhibition of responses to carbachol is much less in bladder than in other tissues such as rat ileum and guinea-pig atria (Madden & Mitchelson, 1975), HC-3's inhibitory effect against responses to acetylcholine might not be apparent except when it is present at very high concentrations.

The action of physostigmine in revealing an inhibitory effect of HC-3 on responses to acetylcholine may be due to an effect of HC-3 on the anticholinesterase activity of physostigimine. With homogenates of ileum, HC-3, in the same concentration as in the bladder experiments, could reduce the effectiveness of physostigmine as an inhibitor of acetylcholine hydrolysis (Table 4). Thus, by reducing the anticholinesterase activity of physostigmine, HC-3 could be inhibiting responses to acetylcholine without being any more effective at antagonizing the action of the available acetylcholine on the postjunctional receptor. That physostigmine releases endogenous acetylcholine or has a direct effect on smooth muscle receptors in the bladder is not supported by present findings.

The action of HC-3 in the presence of DFP in the bladder appears to be due to some effect of DFP on the postjunctional tissue, as HC-3 is more effective as an antagonist of both acetylcholine and carbachol. Although HC-3 reduced the effectiveness of DFP as an inhibitor of acetylcholine hydrolysis in ileal homogenates, this only occurred when HC-3 was added *before* the DFP (Table 5). As the isolated tissue experiments involved addition of HC-3 some time after a 30 min incubation with DFP, HC-3 could not be reactivating the enzyme, since no evidence for such an effect was found in the cholin-

esterase determinations. A series of bisquaternaries related to hexamethonium reduced the binding of DFP with acetylcholinesterase although they did not reactivate the phosphorylated enzyme (Lüllmann, Ohnesorge & Wassermann, 1967; Lüllmann, Ohnesorge, & others, 1971). HC-3 may possess similar properties which would account for the observed effects with both physostigmine and DFP.

Edrophonium ($4{\cdot}1 \times 10^{-5}$ M), although potentiating responses to acetylcholine and nerve stimulation, inhibited responses to carbachol slightly, suggesting some inhibitory action on the postjunctional receptors which was more pronounced at a 10-fold higher concentration. A similar inhibitory effect has been reported on rat atria for both chronotropic and inotropic muscarinic receptors over the same concentration range (Roberts & Konjovic, 1969). That edrophonium ($4{\cdot}1 \times 10^{-4}$ M) reduced the HC-3-induced inhibition of responses to carbachol could possibly be explained by an allosteric interaction between edrophonium and HC-3 on the postjunctional receptor.

Other bisquaternaries such as hexamethonium (Geddes, Bogart & Hamilton, 1974) and hexafluorenium (Scaf, 1971) have been reported to be more effective at inhibiting responses to carbachol than those to acetylcholine or methacholine in guinea-pig gut. Such an action may also be due to a metaffinoid antagonism of the muscarinic receptor by the bisquaternaries.

In conclusion HC-3 has a variety of actions apart from inhibiting choline uptake into cholinergic neurons. It has a postjunctional action at the neuromuscular junction (Bowman & Rand, 1961; Marshall, 1969) a ganglion blocking action (Beck—quoted in Leaders & Pan, 1967) and anticholinesterase activity (Domino, Schellenberger & Frappier, 1968; Hemsworth, 1971). Furthermore, it can inhibit the action of agonists such as acetylcholine or carbachol on the muscarinic receptor to differing degrees and reduce the effectiveness of some anticholinesterases such as physostigmine. When HC-3 is used as a pharmacological tool these possibilities should be borne in mind.

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