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Anticholinergic effects and passage through the intestinal wall of *N*-butylhyoscine bromide

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The effects of *N*-butylhyoscine bromide (Buscopan) were examined on responses of guinea-pig isolated intestine to transmural stimulation and to peristalsis induced by raising the intraluminal pressure. The drug acted rapidly and in low concentration to abolish peristaltic activity and responses to transmural stimulation when applied to the serosal surface of the ileum, but from 100 to 1000 times the concentration was required when it was applied to the mucosal surface. The effects were more persistent after mucosal than after serosal application. *N*-Butylhyoscine bromide was bound to mucus and mucosal material, the ratio of free to bound drug being 2:1. The drug passed through portions of the intestinal wall containing Peyer's patches more rapidly than through portions containing no macroscopically visible lymphoid tissue. The rate of passage through Peyer's patches, but not through other portions, increased with increasing hydrostatic pressure. These findings help to explain why enterally administered *N*-butylhyoscine bromide exerts anticholinergic effects on the gut without producing systemic actions.

Parenteral administration of *N*-butylhyoscine bromide is effective in the treatment of spasm of the smooth muscle of the alimentary tract (Wick, 1951). It also affects other cholinergically innervated structures (Brownlee, Wilson & Birmingham, 1965; Herxheimer & Haefeli, 1966). However, Herxheimer & Haefeli (1966) reported that oral-*N*-butylhyoscine bromide in volunteers had no effect on heart rate, salivary secretion or accommodation, suggesting that it was not absorbed from the gut. It has been said that orally the drug is inactive, even on the gut, in the doses usually administered (20 mg, 4 to 5 times daily) (Herxheimer & Capel, 1963, 1966). On the other hand, Wick (1967) and Pennefather, McCulloch & Rand (1968) produced evidence from animal experiments for the absorption of the drug after enteral administration, and Pennefather & others (1968) showed that it abolished cholinergically-induced responses of the gut.

This paper deals with the effects of *N*-butylhyoscine bromide in blocking cholinergic responses in isolated gut after application to mucosal or serosal surfaces. The passage of the drug through the intestinal wall was also examined. The findings go some way towards reconciling the apparently conflicting observations previously reported.

EXPERIMENTAL

Method

Observations were made on portions of guinea-pig isolated ileum suspended in an organ bath in McEwen solution (McEwen, 1956) bubbled with 5% CO_2 in oxygen, and maintained at 35·5°.

Peristaltic activity of segments of ileum was studied by Trendelenburg's method (see Burn, 1952), intraluminal pressure being measured with a water manometer and longitudinal movements with a frontal lever exerting 1 g tension and giving a 6-fold magnification. Care was taken to ensure that no leakage of fluid occurred from the lumen of the segment of ileum to the McEwen solution surrounding it in the organ bath. The resting intraluminal pressure was raised to 2 to 6 cm of water pressure for periods of 1·5 to 3 min to induce peristaltic activity. The preparation was then allowed to rest for 3 min.

Twitches of the ileum were produced as described by Paton (1957) by transmural stimulation with 50 V pulses of 1 msec duration at a frequency of 0·1 s for periods of 1·5 to 2·5 min. After such a period of stimulation, the preparation was allowed to rest for 2 min. In some preparations, peristaltic activity and responses to transmural stimulation were recorded in alternate periods.

The amounts of *N*-butylhyoscine bromide (Buscopan) referred to in the text are in terms of the salt. The drug was applied to the serosal surface by placing it in the fluid surrounding the preparation. It was applied to the mucosal surface, that is, into the lumen of the intestinal segment, by passing a fine polythene tube through the Trendelenburg cannula so that the tip was within the intestinal sac. *N*-Butylhyoscine in McEwen solution was injected through the polythene tube in sufficient volume to flush out the fluid already present through a T-piece in the tubing connecting the cannula with the reservoir: care was taken to avoid increases in pressure during this procedure. In some experiments, everted segments of intestine were used, in which case the drug was applied to the serosal surface by giving it into the lumen.

Some observations were made on the passage of the drug through circumscribed regions of the wall of portions of intestine. A segment of intestine was slipped over a glass tube of 5 mm external diameter and a selected region of the intestine was located over an oval hole (5 mm × 7·5 mm) in the side of the tube. The segment was then tied in place using double ties to ensure there was no leakage. Drug solutions were passed through this tube and the rate of flow and the hydrostatic pressure could be varied. The segment of intestine tied over the tube was immersed in an organ bath. In some experiments, this organ bath also contained another segment of ileum arranged for recording of responses to transmural stimulation, and this served to detect the drug passing through the selected region of the wall of the perfused segment. In other experiments, samples were taken from the bath surrounding the perfused segments. At the conclusion of each experiment, azovan blue or Congo red solution were perfused through the tube to ensure that there had been no leakage.

The potential difference across the wall of the ileum was measured in segments that were converted to flat sheets by a longitudinal division of the wall. The sheets were rinsed in McEwen solution and laid flat under paraffin. A platinum electrode was applied to the lower surface and a platinum probe was applied to selected portions of the upper surface. The potential was measured on a Tetronix 502A oscilloscope.

Mucus and mucosa were collected by opening a long segment of ileum and gently scraping the mucosal surface with a scalpel blade.

RESULTS

Relative anticholinergic activity of N-*butylhyoscine applied to serosal and mucosal surfaces of the ileum*

The drug added to the fluid bathing the preparation, that is, applied to the serosal surface, abolished responses to transmural stimulation in concentrations of 0·1 to 0·6 μg/ml. Concentrations of 4 to 6 μg/ml were required to abolish peristaltic responses to increased intraluminal pressure. The longitudinal contractions associated with peristaltic activity were abolished earlier and with slightly lower doses of the drug than were the pressure waves due to propagated contractions of circular muscle. The blockade of responses to transmural stimulation and of peristaltic activity by the drug, in the appropriate concentrations, was fully established in 30 to 60 s. Fig. 1 illustrates its effects on circular and longitudinal muscles involved in peristaltic activity.

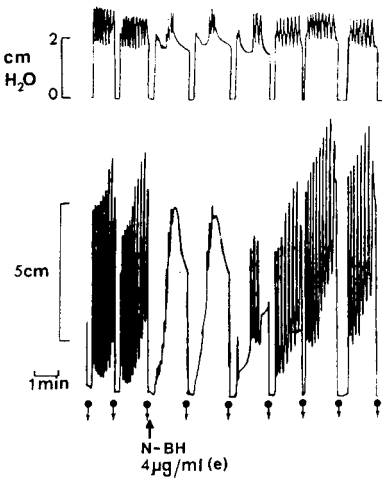

FIG. 1. Blockade of peristaltic activity by *N*-butylhyoscine (N–BH) applied to the external (e), serosal surface of guinea-pig ileum. Upper trace: intraluminal pressure. Lower trace: longitudinal movements. The drum was stopped and the bath washed out after each period of peristaltic activity as indicated by the symbol ⏚.

With doses of the drug that reduced but did not abolish responses to transmural stimulation (0·03 to 0·1 μg/ml), the twitches were reduced in height within 30 s, but then recovered somewhat and reached a constant depressed level within 5 min (Fig. 2A). The effect of the drug was easily removed by one exchange of the bath fluid with fresh McEwen solution.

When *N*-butylhyoscine was applied into the lumen of the ileal sac, that is, to the mucosal surface, much larger concentrations (600 to 800 μg/ml) were required to abolish peristaltic activity and responses to transmural stimulation within 5 min. There was no apparent difference in the concentrations affecting the two types of response (Fig. 3).

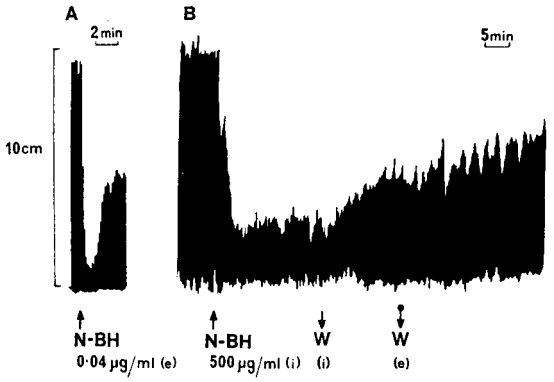

FIG. 2. Effects of *N*-butylhyoscine on transmurally stimulated segments of guinea-pig ileum, in A, applied to the external (e), serosal surface, and in B, applied intraluminally (i) to the mucosal surface. The preparation was washed at W by exchanging the intraluminal fluid (i) or external bath fluid (e) with fresh McEwen solution.

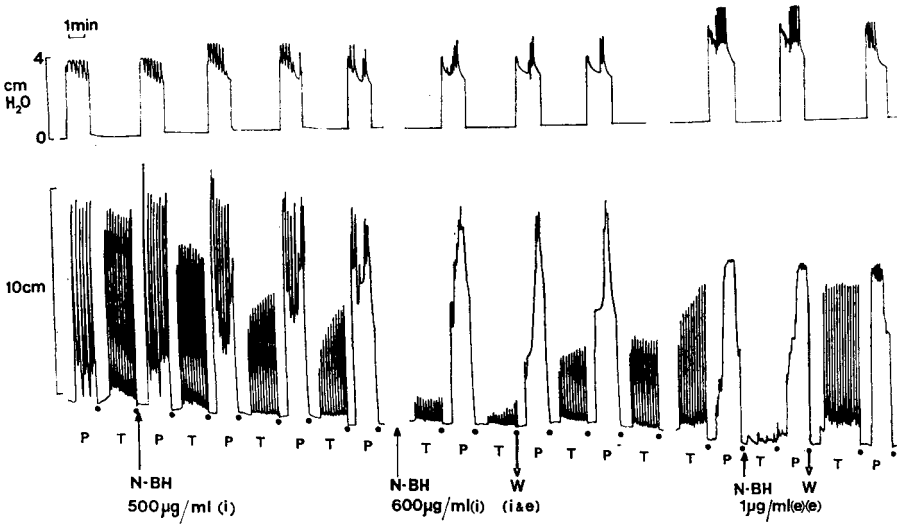

FIG. 3. Effects of *N*-butylhyoscine (N–BH) applied to the internal mucosal surface (i), and to the external serosal surface (e), on responses of guinea-pig ileum to transmural stimulation (T) and to increases in intraluminal pressure (P). The drum was stopped for 2 to 3 min after each period of stimulation as indicated by a dot. The drug-containing solution was washed out at W. *N*-Butylhyoscine caused approximately 50% reduction in responses in a concentration of 500 μg/ml and about 90% reduction on increasing this to 600 μg/ml. The responses gradually recovered after washing out the lumen of the gut (i) and the organ bath (e). Then, 1 μg/ml applied to the external surface abolished responses to transmural stimulation but had no effect on peristaltic activity. Washing out the external fluid resulted in an immediate restoration of responses.

Replacing the drug solution in the lumen with one exchange of fresh McEwen solution either resulted in a slight restoration of responses or failed to cause any restoration. An exchange of the external fluid usually resulted in a slight increase in responses, suggesting that traces of the drug had passed through the wall of the ileal segment (Fig. 2B). With further exchanges of intraluminal fluid, responses to transmural stimulation were restored to a greater extent and more rapidly than was peristaltic activity. Two exchanges of intraluminal fluid with fresh McEwen solution usually resulted in restoration of responses to transmural stimulation to the control

level, but 5 to 6 exchanges of the intraluminal fluid were required before peristaltic activity was restored.

Experiments were made with everted segments of intestine to establish that the difference in anticholinergic potency of *N*-butylhyoscine was in fact due to differences in its penetration when applied to serosal or mucosal surfaces. With everted segments, responses to transmural stimulation and peristaltic activity were obtained. These were abolished by low concentrations of the drug applied intraluminally, that is, to the serosal surface, whereas high concentrations were required to abolish responses when the drug was added to the fluid bathing the preparation, that is, applied to the mucosal surfaces. The effective concentrations for application at each surface of everted segments were of the same order as those found with normal segments.

With doses of the drug applied intraluminally that reduced but did not abolish responses (400–600 μg/ml), both types of responses were equally depressed. In about half of the preparations, the effects developed fully during the course of 5 min and there was no further depression in the following 30 min. However, in the remaining preparations, there was a slow continuing decline in responses throughout the ensuing period of observation (up to 2·5 h). Records from the preparations showing these two types of behaviour are illustrated in Fig. 4. It was noticed that preparations in which there was a slow continuing decline in responses contained at least one Peyer's patch (lymph node) in the wall of the segment. The rate of decline of responses was sometimes increased by raising the intraluminal pressure in inducing peristaltic activity.

Some of the preceding observations led to experiments designed to test the influence of hydrostatic pressure on the passage of the drug through the intestinal wall after intraluminal application to the mucosal surface. In these it was also possible to

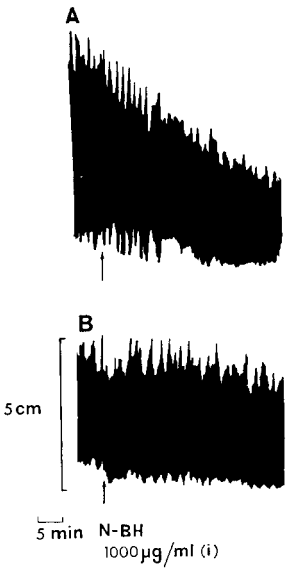

FIG. 4. Effects of *N*-butylhyoscine perfused through segments of ileum in a concentration of 1 mg/ml on transmurally stimulated detector segments in the same bath. In A, the perfused segment contained a Peyer's patch and in B, there was no macroscopically visible lymphoid tissue in the segment.

test whether the drug passed through the wall more rapidly when a Peyer's patch was present. Segments of intestine were slipped over a glass tube and selected portions of the intestinal wall were orientated over an oval hole in the wall of the tube. Various concentrations were circulated through the glass tube. Drug passing through the portion of wall covering the hole in the tube was detected by observing its effect in depressing responses to transmural stimulation in another piece of ileum. When *N*-butylhyoscine was perfused at zero hydrostatic pressure, responses of the detector segment to transmural stimulation were depressed about equally whether or not a Peyer's patch was present. However, when the hydrostatic pressure was increased there was a much greater and more rapid rate of onset of depression of responses in the detector segment when a Peyer's patch was present in the perfused segment, but this did not occur in segments without a Peyer's patch. Fig. 5 illustrates typical results with an internal of concentration of 400 μg/ml. Similar results were obtained with 800 μg and 1 mg/ml.

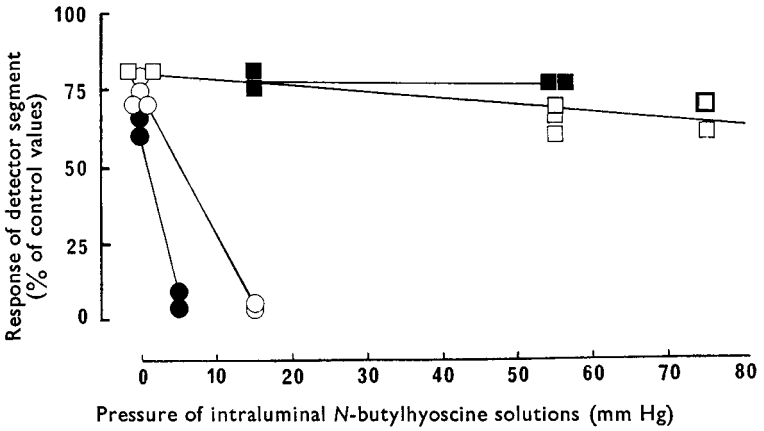

FIG. 5. Effect of *N*-butylhyoscine passing through the wall of perfused segments of guinea-pig gut on responses of transmurally stimulated detector segments. *N*-butylhyoscine in a concentration of 400 μg/ml was applied to the mucosal surface. The perfused segments were in a 10 ml bath. The bath fluid was removed at 5 min intervals and assayed. Results from segments of gut containing a Peyer's patch are indicated by ● and ○, those from segments without macroscopically visible lymphoid tissue are indicated by ■ and □. The open symbols are of segments taken from the caecal end of the gut, and the solid symbols are from the duodenal end. The sensitivity of detector segments was as follows: 80–95% depression, 80–90 ng/ml; 20–40% depression, 20–40 ng/ml.

On careful inspection of the mucosal surface of ileum, it was noticed that there was less mucus over a region containing a Peyer's patch. It had also been noticed that some segments used in the earlier experiments had large amounts of mucus present and these were unusually resistant to the anticholinergic action of the drug applied to the mucosal surface. Therefore, experiments were made in which 0·4 g of mucus and mucosal surface was suspended in 3 ml of solutions containing the drug. The mixture was centrifuged and the supernatant was assayed for anticholinergic activity on transmurally stimulated ileal segments. They were found to have activities corresponding to 60 to 70% of the concentration of *N*-butylhyoscine orginally present, this relation holding good over a concentration range of 10 to 800μg/ml. Therefore, 30–40% of the drug was removed, presumably by binding.

A potential difference across the intestinal wall, the serosal surface being positive with respect to the mucosal surface, would tend to prevent the *N*-butylhyoscine cation, when applied to the mucosal surface, from passing through the wall. An endogenous potential difference of about 2 mV across the wall of 32 day avian ileum was reported by Hudson & Levin (1968). Measurement of transmural potential difference in portions of guinea-pig ileum gave values of 6 to 8 mV. There was no difference between the potential over a Peyer's patch and that over a region macroscopically free of lymphoid tissue. There was no difference between segments taken from jejunal and caecal ends of the ileum.

DISCUSSION

N-Butylhyoscine bromide acts rapidly and in low concentrations in abolishing peristaltic activity and responses to transmural stimulation when applied to the serosal surface of the ileum, but from 100 to 1000 times the concentration is required when it is applied to the mucosal surface. This suggests that there is a substantial barrier to its penetration from the mucosal surface to cholinergic receptors on the smooth muscle in the wall. The drug, being a quaternary nitrogen compound will be virtually insoluble in the lipoid component of the mucosal cell membranes, and this probably represents the most important barrier to the drug. Furthermore, the drug is bound to mucus and mucosal tissue, and this binding may provide a further barrier to passage of the drug into and through the wall. An additional factor providing a barrier to penetration is that *N*-butylhyoscine, being a cation, would be moving against the electrical gradient of the transmural potential. Nevertheless, it can exert anticholinergic effects on the ileum after application to the mucosa providing it is present in a sufficiently high concentration.

However, passage of the drug through the wall, rather than action within the wall is poor, even with high concentrations. It is generally recognized that the absorption of ionized drugs from the intestine is poor and erratic. This applies particularly to drugs such as *N*-butylhyoscine bromide which contain a quaternary group (Levine & Pelikan, 1964). It appears from our studies with guinea-pig ileum that the passage of *N*-butylhyoscine through the wall will depend on the numbers and distribution of Peyer's patches, on the amount and distribution of mucus, on the intraluminal pressure and on the concentration of the drug. The finding that *N*-butylhyoscine passed more readily through the wall of the ileum containing a Peyer's patch than through other regions suggests that the drug may be carried into the general circulation via the lymphatics rather than via the portal system. The increased passage of *N*-butylhyoscine through portions of wall containing a Peyer's patch may be due to the fact that there is less mucus on the surface in these regions, but we have no direct evidence on this point. The binding of the cation *N*-butylhyoscine to mucus may be due to the polyanionic nature of mucus.

Herxheimer & Haefeli (1966) produced evidence that there was insignificant absorption of *N*-butylhyoscine from the gut even after oral administration of 600 mg which is six times the recommended dose. They suggested that it was without action on the gut after oral administration. However, absence of systemic effects does not preclude a local action. In fact, it has now been shown that high concentrations of the drug applied to the mucosal surface of the guinea-pig ileum abolished peristaltic activity and responses to transmural stimulation of cholinergic nerves, but that only small amounts actually passed through the wall of the ileum.

Wick's (1967) evidence that *N*-butylhyoscine is absorbed was based on the findings that LD50 was lower and death occurred much earlier after intraduodenal than subcutaneous administration. Wick also noted that the LD50 after instillation of *N*-butylhyoscine into the stomach was higher than with instillation into the duodenum. He suggested that this was due to decreased gastric peristalsis caused by the drug, and hence a slower passage to sites of absorption in the small intestine. The present observations that a high concentration of the drug decreased peristaltic movements supports Wick's suggestion.

The present findings extend the observations made by Pennefather & others (1968). They demonstrated that *N*-butylhyoscine was absorbed from the gastrointestinal tract as shown by the blockade of cardiovascular responses to vagal stimulation. However, their main finding was that *N*-butylhyoscine, administered into the duodenum, had a much greater antagonistic effect on cholinergically induced responses of the gut than on responses of other effectors. In the light of the present findings, this may be interpreted as a local effect on the gut with relatively little absorption.

The binding of *N*-butylhyoscine to mucus and mucosal tissue may explain the slow return of responses after washing out the drug-containing fluid from the lumen of isolated intestinal segments and the necessity for repeated washing to obtain full recovery of responsiveness, the bound *N*-butylhyoscine being slowly released. Furthermore, it may explain the persistence of action of enterally administered *N*-butylhyoscine as compared with intravenous injections in the experiments of Pennefather & others (1968).

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