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The effect of 6-azauridine on the contractile responses of the isolated ileum of the guinea-pig to drugs and to coaxial stimulation

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Small concentrations of 6-azauridine augmented the contractions of the guinea-pig isolated ileum to acetylcholine, nicotine, histamine, barium chloride and to coaxial stimulation. High concentrations partially inhibited all the responses. After prolonged contact with high concentrations of 6-azauridine, the anti-spasmogenic effect persisted long after washing out the compound. The acetylcholine output did not change under these conditions. Comparison of the actions of 6-azauridine with those of papaverine is made and the possible mechanisms of action are discussed.

THE nucleoside 6-azauridine is known for its inhibitory activity on some animal tumours (Jaffe, Handschumacher & Welch, 1957; Šorm & Keilova, 1958). This activity results from its phosphorylation *in vivo* to 6-azauridine monophosphate which in turn inhibits orotidylic acid decarboxylase (Habermann & Šorm, 1958; Handschumacher & Pasternak, 1958).

In previous papers from this laboratory (Janků, Kršiak, Volicer, Čapek, Smetana & Novotný, 1965a; Janků, Kršiak, Novotný, Volicer & Čapek, 1965b), an analysis was made of the effects of this nucleoside and its amino-analogue, 6-azacytidine, on the central nervous system. We now report the effects of 6-azauridine on the isolated ileum of the guinea-pig. Except for the work of Smith (1964), who found that the effects of some 6-azapyrimidine derivatives on smooth muscle was relaxant, these agents have not been studied.

Experimental

MATERIAL AND METHODS

Terminal isolated guinea-pig ileum bathed in modified Krebs-Henseleit solution (Eccles, 1952) at 37°, gassed with a mixture of 95% oxygen and carbon dioxide 5% was used. 6-Azauridine (Spofa) was tested on the ileum in two ways. (1) A single dose, diluted in Krebs solution with the pH adjusted when necessary with sodium bicarbonate, was added to the bath. (2) When concentrations of 6-azauridine from 2 to 8 mM were used, an equivalent amount of sodium chloride was omitted from the Krebs solution and replaced by the drug. This enabled relatively high concentrations of drug to remain in contact with the tissue for long periods without osmotic change.

The effects of adding 6-azauridine by methods (1) and (2) on the response to spasmogenic drugs were tested as follows. A dose of spasmogen

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causing approximately 40% of the maximal response was determined. This was referred to as the standard dose, the standard response being considered 100%. The size of the response in the presence of 6-azauridine was measured as a percentage of this standard response.

In other experiments, ileum taken at least 15 cm above the ileocaecal valve, was stimulated by the method of Paton (1954). In some cases the acetylcholine output from the stimulated ileum was measured in the presence of eserine salicylate, 5×10^{-6} g/ml. The bioassay was performed on guinea-pig ileum in Krebs solution containing 12·5 μg/litre eserine salicylate and 6 μg/litre morphine hydrochloride. The ileum was allowed to equilibrate with this solution for 45 min to allow for complete inactivation of the cholinesterases (Paton, 1957). The acetylcholine collections were then made in the following sequence.

(i) The ileum was allowed to rest for a period of 10 min and the acetylcholine released spontaneously into the bathing fluid was estimated.

(ii) The preparation was stimulated for 10 min by means of supermaximal rectangular pulses of 400 μsec duration and a frequency of 1 shock/10 sec and the bath fluid again analysed for its acetylcholine content.

(iii) A further 10 min spontaneous output was estimated.

(iv) The ileum was bathed in Krebs solution containing 20 mM 6-azauridine for 45 min and the ileum was stimulated continuously throughout this period. No collections were taken for assay during this time.

(v) The Krebs solution containing the nucleoside was then replaced by normal Krebs solution and the preparation was washed repeatedly for 10 min.

(vi) Estimations were then made of the spontaneous and stimulated outputs of acetylcholine for five 10 min periods: spontaneous, stimulated, spontaneous, stimulated, spontaneous.

Parallel control experiments were made which were identical with the test experiments with the exception that no 6-azauridine was present in the Krebs solution.

Results

EXPERIMENTS WITH SPASMOGENIC DRUGS

Experiments in which single doses of 6-azauridine were added to the bath revealed that 200–400 μg/ml of the drug increased the responses to nicotine, acetylcholine, histamine and barium chloride. A tenfold increase in the concentrations of 6-azauridine produced a slight increase in the tone of the preparation and the responses to the agonists were enhanced initially, but later markedly reduced.

Similarly, after 6-azauridine had been in contact with the ileum for a long time two effects were observed. The drug, 2 mM, elicited either a sustained or transient increase in the agonist responses or, as was seen in most of the experiments, reduced the contractions. Both effects were more marked after 4 mM of 6-azauridine (Fig. 1) whereas after 8 mM the inhibitory effect prevailed. Dose-response curves in the presence of 8 mM 6-azauridine are shown in Figs 2 and 3. The inhibitory effect against all

spasmogenic drugs developed gradually within the time of contact of the preparation with 6-azauridine. Furthermore, this inhibition of agonist contractions was irreversible after replacing 6-azauridine solution with Krebs solution (Figs 1, 2 and 3). In those experiments in which the drug

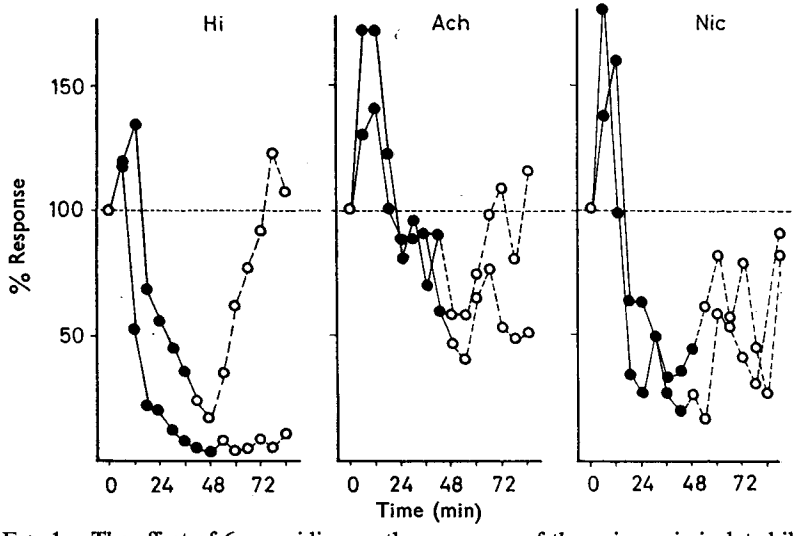

FIG. 1. The effect of 6-azauridine on the responses of the guinea-pig isolated ileum to standard doses (i.e. doses causing a contraction 40% of maximal) of histamine (Hi), acetylcholine (Ach) and nicotine (Nic) in the presence of 4 mM 6-azauridine, (●—●) and after replacing by normal Krebs solution (○ - - - ○). The responses are expressed on the ordinate as percentage of the response before adding 6-azauridine. The time of introducing the drug into the bath is indicated by zero on the abscissa.

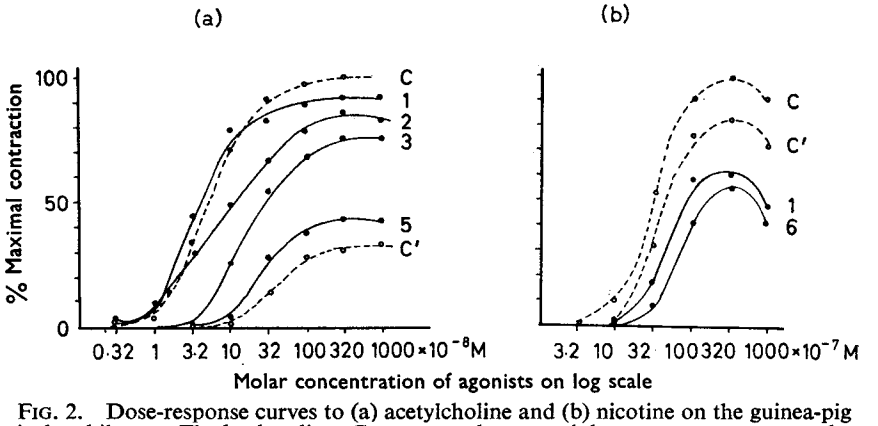

FIG. 2. Dose-response curves to (a) acetylcholine and (b) nicotine on the guinea-pig isolated ileum. The broken lines C represent the normal dose-response curves to the two agonists. The continuous lines (1) represent the responses of the agonists following treatment with 8 mM 6-azauridine. The inhibitory action of the drug became more marked with time so that subsequent dose-response curves (curves 2, 3 and 5 for acetylcholine, curve 6 for nicotine) were more depressed. On washing out the 6-azauridine the inhibition was found irreversible for acetylcholine (C') and partly reversible for nicotine (C').

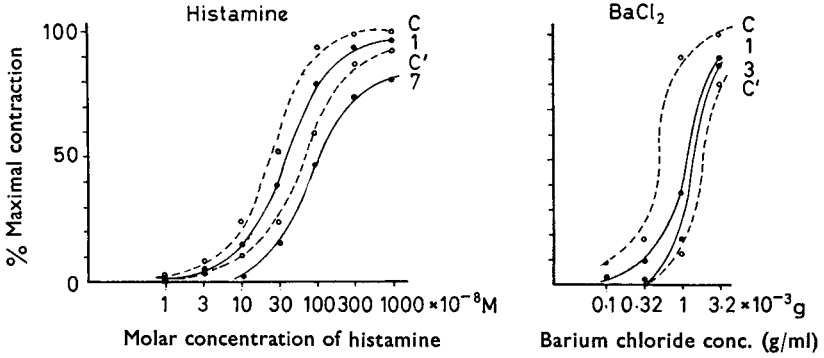

FIG. 3. Dose-response curves for histamine and barium chloride ($BaCl_2$) on the guinea-pig isolated ileum before adding 6-azauridine to the bath broken line C). The continuous lines (1) represent the response of the agonists following treatment with 8 mM 6-azauridine. The inhibitory action of the drug became more marked with time so that subsequent dose-response curves (curve 7 for histamine, curve 3 for $BaCl_2$) were more depressed. On washing out the 6-azauridine the inhibition was irreversible for $BaCl_2$ (C') and partly reversible for histamine (C').

(2 mM) caused a potentiation of the agonist responses, after washing it out, the responses were smaller than those obtained in the control period.

EXPERIMENTS WITH COAXIAL STIMULATION

The effect of 6-azauridine on the ileal responses to coaxial stimulation was dose dependent. Small concentrations potentiated the responses, somewhat higher concentrations caused a transient inhibition, while even higher concentrations produced sustained inhibition (Fig. 4), which never fully recovered after washing out the drug. But in control experiments prolonged electrical stimulation was not accompanied by a decrease in the height of twitches during comparable time intervals (see Fig. 5). Experiments in which part of the sodium chloride in the Krebs solution was replaced by an equivalent amount of 6-azauridine indicated that comparatively higher concentrations (20 mM) were necessary to produce marked pharmacological effects (Fig. 5). With the 20 mM dose, a gradual inhibition developed preceded occasionally by a transient increase in the

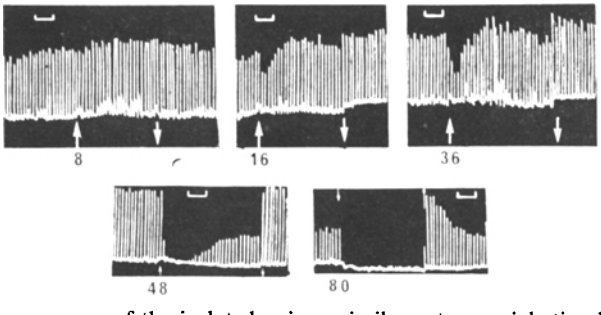

FIG. 4. The response of the isolated guinea-pig ileum to coaxial stimulation in the presence of increasing concentrations of 6-azauridine. The arrows indicate the addition (↑) and removal (↓) of the compound. The concentrations of the drug are in g/ml × 10^{-4}. Time scale: 1 min.

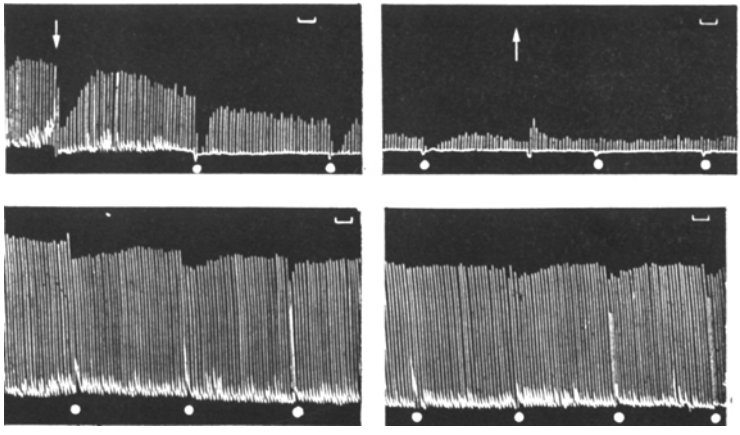

FIG. 5. The responses of the isolated ileum of the guinea-pig to coaxial stimulation during contact (45 min) with 20 mM of 6-azauridine (upper record). 6-Azauridine was present in the bath for the duration between the arrows. The lower record shows a parallel control experiment in which no 6-azauridine was added to the bath. Dots indicate points where the bath fluid was exchanged. Time scale: 1 min.

height of twitches. This inhibition was long-lasting and persisted after washing out the nucleoside. Increasing the concentration of 6-azauridine above 20 mM enhanced the inhibition.

On estimating the acetylcholine released spontaneously and by coaxial electrical stimulation, no significant difference was detected for the outputs obtained before and after a prolonged period of treatment with 20 mM of 6-azauridine. In the parallel control experiments there was a slight decrease of the acetylcholine output within the duration of experiments (Fig. 6).

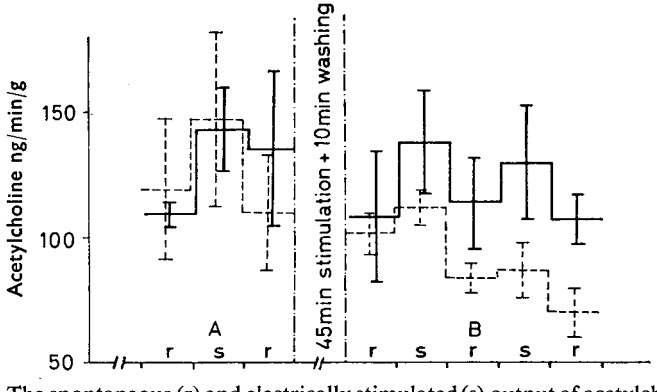

FIG. 6. The spontaneous (r) and electrically stimulated (s) output of acetylcholine from the guinea-pig isolated ileum collected over successive 10 min periods (abscissa). The continuous line indicates the mean outputs from three preparations before (A) and after (B) the 45 min contact with 20 mM 6-azauridine. The broken line indicates the mean outputs from three control preparations over comparable time intervals. The standard errors are also included. The stimulation parameters were: frequency 1 shock/10 sec; duration 400 μsec; voltage supramaximal. Time scale: 10 min.

Discussion

The effects of 6-azauridine on smooth muscle preparations have not been studied extensively. Smith (1964) demonstrated a relaxant action of 6-azauridine diphosphate and high doses of uridine and uracil on the goldfish intestine. Our experiments have confirmed the antagonistic actions of 6-azauridine to nicotine observed in previous experiments *in vivo* (Janků & others, 1965a). However, the responses to other spasmogenic drugs, acetylcholine, histamine and barium chloride, were also blocked by the same concentrations of 6-azauridine as those used to block the nicotine responses. On the other hand, it was possible to detect two distinct components of action on the isolated ileum of the guinea-pig. The first component had a rapid onset of action, and caused an immediate increase or decrease of the height of the responses observed. The second component developed slowly and remained even after removing the 6-azauridine from contact with the preparation. This component was characterised by a gradual diminution of the responses.

It is difficult to believe that the rapid changes in the reactivity of the preparation are due to the same biochemical mechanism which is also responsible for the cytostatic effects of 6-azauridine. The drug is highly ionised at physiological pH (pKa = 6·7) and therefore it hardly penetrates biological membranes, as indicated by its poor penetration of the blood-brain barrier (Habermann & Šorm, 1958; Cardoso & Symmes, 1961). We believe that the rapid component of 6-azauridine's action may perhaps be induced by a direct action on the membrane surface. The biochemical mechanism by which it exerts its action on tumour cells may be considered only in connection with the second component, which develops gradually and persists long after contact of the tissue with the nucleoside is discontinued. This effect might be dependent on the penetration of some 6-azauridine into the cells and its subsequent transformation to 6-azauridine monophosphate.

Like papaverine (Harry, 1962) 6-azauridine did not affect the acetylcholine output of the ileum, although its effects differed in that they were not readily reversible. It is concluded that the drug acts directly on the smooth muscle cells.

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