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Observations on Dopamine Receptor Antagonists and Gastric Ulceration Associated With Experimental Anorexia Cachexia

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WILLIS, G. L., M. SLEEMAN, G. BRODIE AND G. C. SMITH. *Observations on dopamine receptor antagonists and gastric ulceration associated with experimental anorexia cachexia.* PHARMACOL BIOCHEM BEHAV 31(1) 69–73, 1988.—Gastric ulceration is frequently reported to occur in tumour-bearing animals and man, even when such tumours are not associated with organs of digestion. That central and peripheral dopamine (DA) containing neurones may be relevant to this phenomenon, is supported by the fact that the DA receptor antagonists domperidone (0.1 and 0.05 mg/kg) and pimozide (0.1 mg/kg) were observed to prevent gastric ulceration commonly reported in rats bearing the Walker 256 carcinosarcoma. Daily administration of these drugs prevented the formation of ulcers similar to those observed in vehicle-treated animals. These results demonstrate that DA neurone function is important in the formation of gastric ulcers in tumour-bearing animals and suggest that such compounds may be useful in cancer management.

Domperidone Pimozide Tumour-induced gastric ulceration

IT has been reported on several occasions that experimental animals bearing tumours suffer from severe gastric ulceration. The occurrence of such ulceration is not dependent upon either the type or location of a tumour [1]. Similar observations have been made clinically: gastric ulceration has been reported in patients with malignant tumours of various organs other than organs of digestion including the lung and pancreas [3,20].

In a series of recent studies in which we were examining the orexigenic potential of various drugs (unpublished observations), we made the observation that dopamine (DA) receptor antagonists prevented the formation of gastric ulcers in rats bearing the Walker 256 carcinosarcoma. The involvement of the central nervous system in the aetiology of gastric pathology is widely acknowledged [13]. Experimental lesions of the hypothalamus and other limbic areas have been shown to induce gastric ulceration in a variety of species and these are similar to ulcers induced by experimental stress procedures [14]. Investigations of the cytoprotective potential of pharmacological agents with various methods of ulcer induction have further highlighted the critical and perhaps consistent involvement of catecholamines (whether central or peripheral systems) in ulcer formation [14].

The object of the present paper is to report that when central and peripheral DA antagonists were employed to increase food intake, tumour associated gastric ulceration was not observed.

METHOD

Fifty-seven Sprague Dawley rats ranging in weight from 200–250 g were housed individually in wire mesh cages in a room where the ambient temperature was maintained at 22±2°C. There was a 12 hr light/dark cycle used with lights on at 0700 hr. Powdered rat chow (Clarke King: Melbourne) was made available from spillproof dishes (spillage <1 g/day).

All animals were placed on ad lib food and water for at least 5 days prior to commencing the experiment in order to allow food and water intake to stabilise. After this time 50 of the animals were injected subcutaneously in the nape of the neck with 5×10^6 cells of the Walker 256 carcinosarcoma cell line. This suspension was taken from the 1st in vivo passage from the frozen stock originally obtained from the CSIRO, North Ryde, New South Wales. The tumour was aseptically removed and a cell suspension made by a pronase digestion process [5]. Cell viability was estimated by eosin stain exclusion and was appropriately diluted with Dulbecco's modified Eagle's medium (C.S.L. Melbourne) with penicillin (100 U/ml) and streptomycin (100 μg/ml) added prior to injection.

TABLE 1

THE EFFECT OF DRUG TREATMENT AT TIME OF SACRIFICE ON STOMACH CONTENTS AND GASTRIC ULCERATION FOR W256 TUMOUR-BEARING ANIMALS

Treatment	N	Stomach Contents (g) % S.E.M.	Observations
Domperidone 1 (0.1 mg/kg, twice daily)	(9)	3.69 ± 0.36*	no ulceration
Domperidone 2 (0.1 mg/kg, once daily)	(9)	2.25 ± 0.65*	no ulceration
Domperidone 3 (0.05 mg/kg once daily)	(8)	3.56 ± 0.99†	no ulceration
Pimozide 1 (0.1 mg/kg once daily)	(9)	3.47 ± 0.44*	no ulceration
Vehicle (tartaric acid 0.1 N)	(8)	0.76 ± 0.21	severe ulceration in glandular region in 50% of cases
Food Deprived	(7)	0.48 ± 0.08	no ulceration but mucosal inflammation

*$p<0.005$; †$p<0.01$; comparison with vehicle treatment.

On the seventh day after tumour implantation DA receptor antagonists were injected IP in the following doses: domperidone (Janssen) 0.1 mg/kg twice daily, 0.1 mg/kg once daily and 0.05 mg/kg once daily and pimozide (Janssen) 0.1 mg/kg once daily. A group of 10 tumour-bearing (TB) animals served as controls and were injected with vehicle for the duration of the injection regime. Injections were continued up to and including day 15 after tumour implantation. To determine the possible effect of food deprivation on ulcer formation a group of 7 animals were bodyweight matched and food intake matched to the vehicle-injected TB animals for the duration of the experiment. Bodyweight and food and water intake were measured during the 15 days of experimental manipulation between 1000 hr and 1200 hr each day.

At the end of this time animals were rapidly decapitated and their trunk blood collected and centrifuged and the plasma decanted and stored at −70°C until it could be assayed for prolactin by the method described previously [17]. At the time of decapitation tumours were removed and weighed. Tumour growth for a fixed number of injected cells is assumed to be exponential and an equation was derived using a minimum squared error curve fitting analysis. Using this method measurement of tumour weights at the end of the experimental period allowed calculation of daily tumour weights. Individual net bodyweight was found by subtraction of the calculated tumour weight for each animal on each day. The stomachs were also removed, cut around the greater curvature and stretched out on a cork board, assessed for presence of ulceration and then photographed. Ulceration was defined as any break or discontinuity in the gastric mucosa [13]. Stomach contents were removed and then weighed to give an indication of short-term food intake at the time of sacrifice.

Planned comparisons were performed for days 7, 11 and 15 representing the beginning, middle and end of drug treatment. Further analysis consisted of repeated measures analysis of variance and *t*-tests as indicated in the text. For animals in which the tumours did not grow all measurements were excluded from analysis in the study.

RESULTS

As shown in Table 1, gastric ulceration which usually occurs in untreated TB animals was not seen in any of the TB animals treated with domperidone or pimozide. Vehicle-treated TB animals in this experiment exhibited severe ulceration of the glandular region of the stomach (Fig. 1, 50% of cases). In all drug-treated TB animals no mucosal damage was evident. The deprived food intake matched group of nonTB animals did not show any ulceration. Mucosal inflammation was observed in some of these deprived animals.

Food intake (Fig. 2) was significantly reduced during the course of drug treatment in all domperidone- and pimozide-treated animals (two-way repeated measures ANOVA, $p<0.001$ in all cases). Planned comparisons revealed that only the 0.1 mg/kg (×2) and 0.05 mg/kg doses of domperidone caused a significant decrease in food intake on day 7. Food intake in all drug-treated animals was not signifi-

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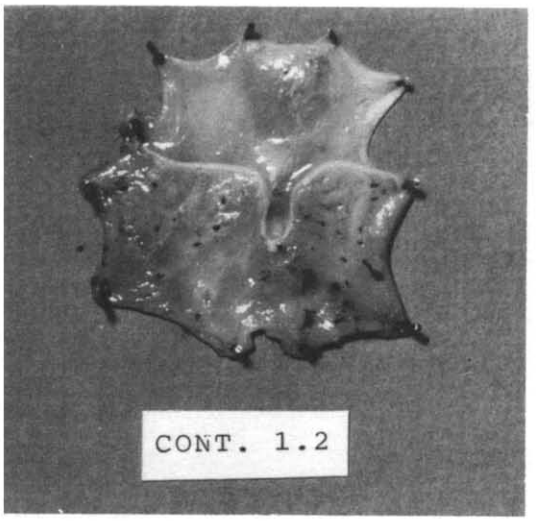
FIG. 1. Representative stomachs from animals treated with vehicle (CONT 1.2), domperidone 0.1 mg/kg×2/day (DOMP 1.5); domperidone 0.1 mg/kg/day (DOMP 2.4); domperidone 0.05 mg/kg/day (DOMP 3.2); and pimozide 0.1 mg/kg/day (PIM 4.5) bearing the W256 tumours and a food intake matched nontumour-bearing animals (DEP 5.2).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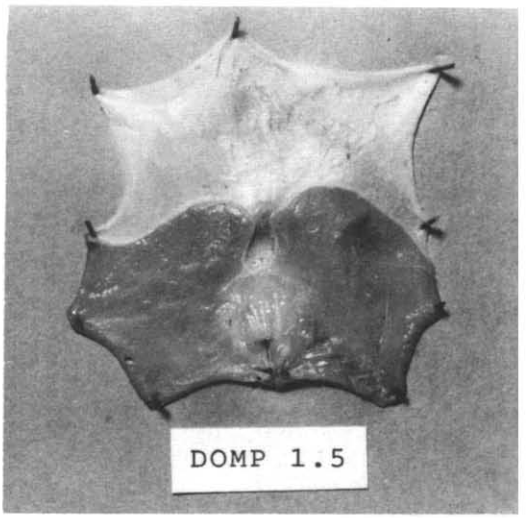
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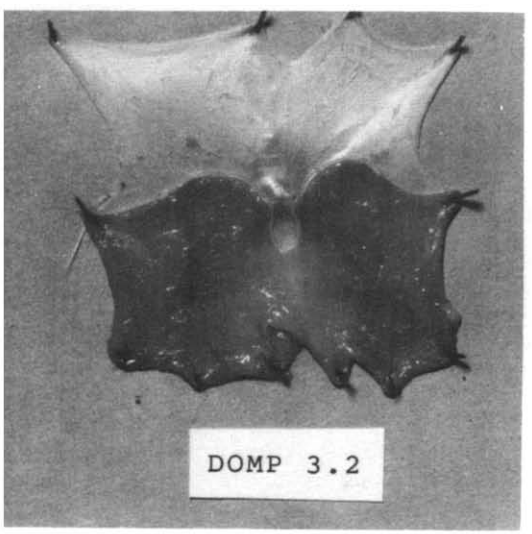
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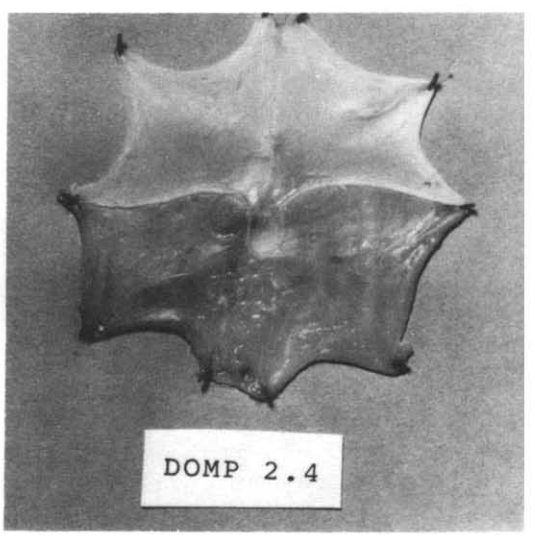
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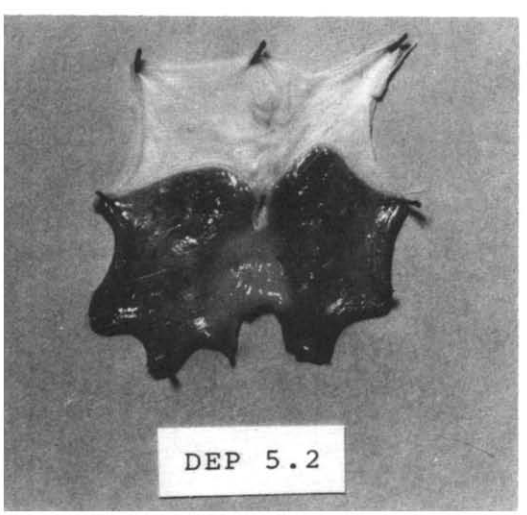
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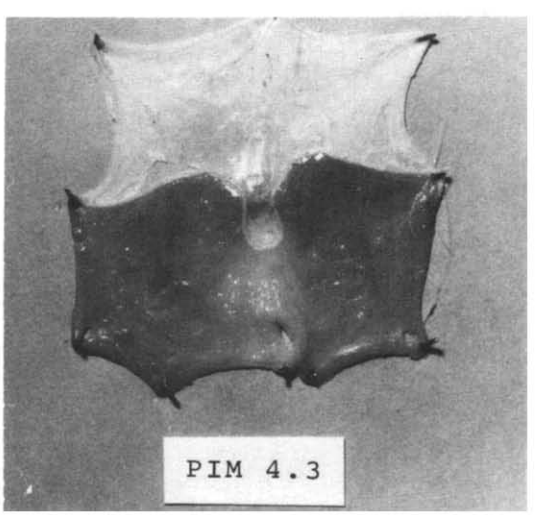
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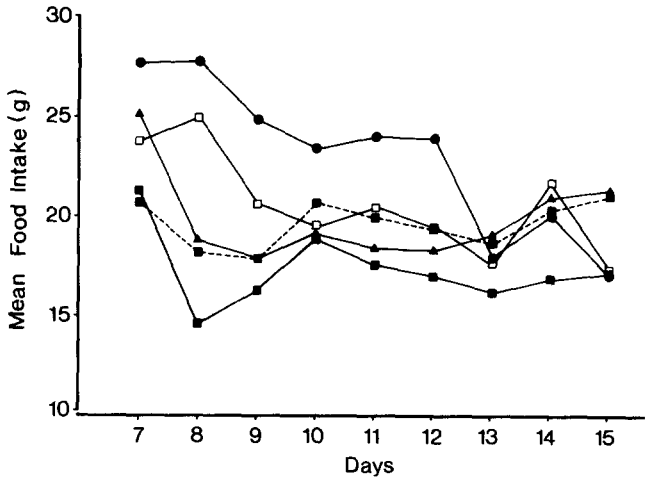

FIG. 2. The effect of drug treatment on food intake of W256 tumour-bearing animals. Vehicle (tartaric acid 0.1 N) ●—●; Domp 0.1 mg/kg×2/day ■—■; Domp 0.1 mg/kg/day □—□; Domp 0.05 mg/kg/day ■- - -■; Pim 0.1 mg/kg/day ▲—▲.

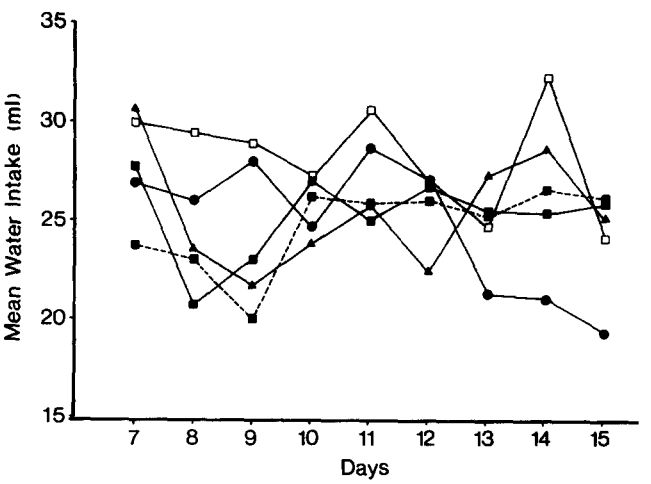

FIG. 3. The effect of drug treatment on water intake of W256 tumour-bearing animals. Vehicle (tartaric acid 0.1 N) ●—●; Domp 0.1 mg/kg×2/day ■—■; Domp 0.1 mg/kg/day □—□; Domp 0.05 mg/kg/day ■- - -■; Pim 0.1 mg/kg/day ▲—▲.

cantly different to that of vehicle-injected controls on days 11 and 15.

Water intake was variable during the 9 days of observation after injection of domperidone or pimozide (Fig. 3). There were no significant effects observed on water intake when planned comparisons were employed for representative analysis of days 7, 11 and 15. Analysis of stomach contents revealed that there was significantly more food in all drug treatment groups at the time of sacrifice than after vehicle treatment (Table 1).

DISCUSSION

The results from the present experiment indicate that treatment with the DA antagonists domperidone and pimozide prevents the gastric ulceration observed in TB animals. This ulceration cannot be attributed simply to the reduced food intake of anorectic TB animals because it is not seen in food intake matched control animals. In earlier experiments Baillie *et al.* [1] reported that the W256 cell line produced ulcers which were restricted primarily to the secretory mucosa as opposed to deprivation-induced ulceration which is found mainly in the forestomach. The ulceration detected in the W256 carcinosarcoma-bearing animals in the present study was also limited primarily to the secretory region.

Findings from our analysis of stomach contents in the present study add further support to the contention that food intake may be stimulated during the latter stages of anorexia for the two drugs employed. At the time of sacrifice a significantly greater mass of food was present in the stomach for all drug-treated animals. In consideration of the gastrokinetic activity which DA antagonists possess [21], we would predict that a stimulation of gastric motility would result in reduced stomach contents in animals treated with these drugs. In fact, this has been reported in anorexia nervosa patients treated with domperidone [15]. It is likely, therefore, that the increase in stomach contents seen after treatment with these drugs was due to increased food intake and is consistent with the trend observed during the 13–15th day of observation (Fig. 2).

Some DA antagonists have been investigated in the treatment of stress-induced ulceration but appear to provide minimal protection [13,16]. In fact there are reports indicating that they may even facilitate ulcer formation [18]. Alternatively, several DA agonists have been reported to exert gastroprotective effects [7, 13, 16]. Apomorphine, bromocryptine, d-amphetamine and methylphenidate have all been used to block stress-induced ulceration in animals. Unfortunately, the mechanism by which this occurs is unknown.

In view of the reduced gastric ulceration which occurs in lateral hypothalamic- (LH) lesioned animals on restricted feeding [6], it may be inferred that the reduced food intake which such lesions produce may be the key element in prevention of ulceration. Results from the present study are in agreement with such a finding in that reduced food intake and bodyweight were seen initially in all drug-treated animals. However, in view of the fact that food deprivation has also been used to promote ulcer formation in various paradigms, the drug-induced reduction in food intake observed in the present study cannot account for the reduced ulceration seen here.

The results of the present study are somewhat conflicting with previous reports in that DA receptor antagonists often enhance gastric ulcer formation rather than prevent it. Possibly to account for this difference, it should be noted that the treatment employed in the current study is a long-term, low dose regime while previous work has focussed mainly on acute, high dose treatments [7, 13, 16, 18]. It has been reported that such long-term regimes can produce DA receptor hypersensitivity which facilitates various types of behavioural recovery [8], but whether a similar mechanism is operating in our treatment regime to reduce ulceration is not possible to determine.

Hyperprolactinaemia has been employed in some studies in an attempt to exert a cytoprotective effect on gastric mucosa and thereby prevent stress-induced ulcer formation [4]. It has been postulated that such an effect is mediated through

central DA transmission and peripheral prostaglandin mediated mechanisms. Since the DA antagonists employed in the present study can produce significant alterations of prolactin activity by affecting DA function in the hypothalamus [11], it stands to reason that this mechanism may be involved in the observed effect. However, the long-term plasma prolactin levels were not altered by this treatment. It is possible that prolactin activity was altered during the first few days of DA antagonist administration and that this was the critical factor in preventing the gastric ulceration. This possibility is currently under investigation.

Gastric ulceration has been observed in patients with tumours which are not linked directly with gastrointestinal function. Patients with tumours of the lung, for example, often present with a high incidence of gastric ulceration [3,20]. The important implication of these observations is that domperidone may be useful in the prevention and treatment of this ulceration. In view of its limited capacity to cross the blood-brain barrier it thereby lacks the extrapyramidal side effects that have been noted with centrally acting DA antagonists [10]. This drug is also known to possess powerful antinauseant properties [12] and has been used recently in trials to block nausea and vomiting associated with chemotherapy [2,5]. This combination of effects strongly suggests that it may be effective in treating several aspects of anorexia cachexia.

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