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# Interactions of aminoglutethimide with analgesics using antinociceptive tests in mice

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The action of aminoglutethimide in alleviating bone pain in women with metastatic breast cancer may be due to either an inherent analgesic effect or an interaction with other analgesic drugs. These possibilities have been investigated in mice by conventional antinociceptive tests. In the abdominal constriction test, aminoglutethimide alone had a dose-related antinociceptive activity. A low dose (which had no pharmacological activity) when co-administered with an effective sub-maximal dose of the analgesic, potentiated the effects of the non-steroidal anti-inflammatory drugs (NSAIDs) tested. In the tail immersion test, aminoglutethimide was inactive and did not enhance the antinociceptive activity of the centrally acting analgesics. As cytochrome P450-dependent routes which are inhibited by aminoglutethimide are not involved in the metabolism of the NSAIDs studied, an interaction at the drug metabolism level cannot explain these results. The NSAID-like activity of aminoglutethimide provides some evidence that the drug's mode of action may involve more than the suppression of oestrogen biosynthesis.

Aminoglutethimide (AG) was initially introduced as an anticonvulsant in 1960 but was withdrawn after it was found to produce adrenal insufficiency. It inhibits an early step in adrenal steroid biosynthesis—the conversion of cholesterol to pregnenolone (Gale 1982; Harris 1985). This inhibition can be overcome by ACTH, so replacement doses of hydrocortisone are also given clinically to block the feedback effect.

Currently the drug is used in the therapy of oestrogen-dependent breast cancers in postmenopausal women, in whom it acts by inhibiting peripheral aromatase by 95–98% (Santen et al 1978; Santen & Wells 1980), thus preventing the conversion of androgens to oestrogens (Harris et al 1983a). Breast cancers frequently metastasize to bone, resulting in an intense pain which has been treated by a number of agents including non-steroidal anti-inflammatory drugs (NSAIDs) (Powles et al 1980). It has been reported that therapy with AG results in a decrease of pain in patients with bone metastases (Santen et al 1982; Harris et al 1983b) who are already taking NSAIDs. AG is also effective in alleviating the bone pain suffered by male patients with advanced prostatic cancer (Sanford et al 1976; Rostom et al 1982)—a phenomenon which is not explained completely by its effects on steroid biosynthesis.

Since AG has a beneficial effect in alleviating bone pain in cancer patients, we have used conventional antinociceptive tests to investigate the possibility that AG interacts with analgesics in mice.

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## *Materials and methods*

*Animals.* Male albino mice (Triangle Mousery, Newton Abbott, Devon, UK), GB1 variants of an ICI-derived strain, 18–22 g, were housed in groups of 12 and allowed at least 5 days to acclimatize. They were maintained on a natural light-dark cycle, at 22 °C ± 1 °C, and had free access to food (breeding diet 675, Grain Harvesters Ltd., Kent) and tap water. In all experiments food was withdrawn the previous night and water 2 h before experimentation. Animals were used only once.

*Drugs and chemicals.* Aminoglutethimide (Ciba-Geigy, Horsham, UK), dextromoramide tartrate (MCP Pharmaceuticals Ltd, West Lothian, UK), ibuprofen (Boots Company, Nottingham, UK), mefenamic acid (Warner-Lambert/Park-Davis Research Division, Pontypool, UK), paracetamol (Sterling Organics Ltd, Northumberland, UK) were gifts. Acetic acid (BDH, Poole, UK), aspirin (Boots Company Ltd, Nottingham, UK), carboxymethylcellulose (ICI Organics Division Ltd, UK), dihydrocodeine tartrate (DF 118; Duncan, Flockhart & Company Ltd, London, UK), indomethacin, Tween 80 (Sigma, Poole, UK), morphine hydrochloride (MacCarthys, Bristol, UK) were purchased.

All concentrations refer to the compounds as received. The drugs were given orally in a suspension of 0·1% w/v carboxymethylcellulose/0·1% v/v Tween 80, 0·1 mL/10 g weight. Acetic acid was administered intraperitoneally (i.p.) as a 3% v/v solution in deionized water.

*Abdominal constriction test.* The test described by Koster et al (1959) was employed. Acetic acid (3%, 0·1 mL/10 g body weight) was injected i.p. and the total number of abdominal constrictions elicited in the 30 min following the injection was counted (n = 16). Initial experiments with standard analgesics, given orally as a suspension (in 0·1% w/v carboxymethylcellulose/0·1% v/v Tween 80, 0·1 mL/10 g weight) 45 min before the i.p. irritant, were made to select an effective sub-maximal dose that could be given in combination with AG. The interactant dose of analgesic was such that it produced approximately 40% protection against the acetic acid-induced writhes (see Fig. 1). The dose of AG chosen (10 mg $kg^{-1}$) did not exhibit marked antinociceptive activity calculated using the following formula:

% antinociceptive effect = (a − b) × 100/a where a = score of control (vehicle-treated) group, b = score of drug-treated group.

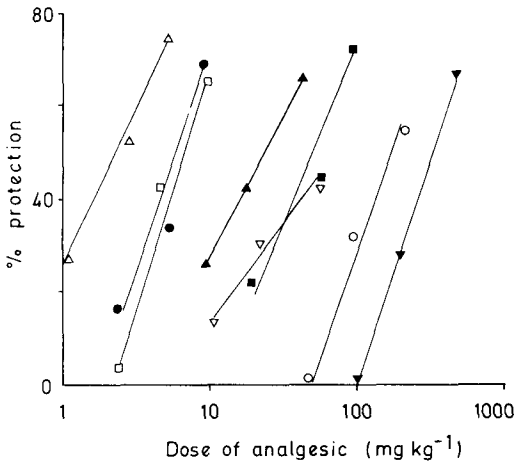


FIG. 1. Log dose-response relationship for the analgesics in the abdominal constriction test. Animals were pretreated with standard analgesics, given orally as a suspension (in 0·1% w/v carboxymethylcellulose/0·1% v/v Tween 80, 0·1 mL/10 g weight) 45 min before challenge with the intraperitoneal irritant (3% v/v acetic acid) and the total number of abdominal constrictions elicited in the 30 min following injection of the irritant were counted. The interactant dose of the analgesic chosen was such that it produced 30–50% protection against the abdominal constrictions (i.e. an effective sub-maximal dose). Drugs used: aspirin (○), dextromoramide (●), dihydrocodeine (□), ibuprofen (▲), indomethacin (▽), mefenamic acid (■), morphine (△), paracetamol (▼).

*Tail immersion test.* AG (10 mg kg$^{-1}$) was also tested in the tail immersion test at 48 °C (Sewell & Spencer 1976), alone and in combination with dihydrocodeine, indomethacin, morphine and paracetamol at the same dose as used in the abdominal constriction test. Animals were placed in rigid plastic ventilated tubes (n = 20) and the nociceptive sensitivity monitored at 20 minute intervals for 120 min. A 15 s 'cut-off' time was imposed for all animals that failed to respond to the stimulus to prevent tissue damage. The antinociceptive effect was calculated as described by Sewell & Spencer (1976).

*Plasma levels of AG.* Plasma levels of AG were measured by reverse phase high performance liquid chromatography after dichloromethane extraction, 1 h after oral administration, the time that peak plasma levels of AG are attained after oral administration (Abusrewill et al 1986). For this reason, mice were pretreated (either with analgesic, AG, or both) 45 min before being tested in the abdominal constriction test. Blood was collected into heparinized tubes by cardiac puncture under ether anaesthesia, and centrifuged at 300*g* for 15 min to produce the plasma.

*Analysis of the data.* The data from the constriction test were analysed using a linear model which assumes a Poisson distribution. This indicated whether or not an interaction was present. Percentage antinociceptive activity (protection against abdominal constrictions) was also calculated. For the tail immersion test, the data were analysed using the Mann-Whitney U-Test.

## *Results*

*Selection of dose of AG.* Initial experiments showed that AG alone had a protective effect (Table 1), particularly at doses over 20 mg kg$^{-1}$. At the higher doses the animals were physically sedated which may have contributed to the protective effect seen. The dose used subsequently was 10 mg kg$^{-1}$ since its antinociceptive effect was minimal.

*Plasma levels of AG.* The 10 mg kg$^{-1}$ dose produced plasma levels of 2 μg mL$^{-1}$ 1 h after administration which correlate with the levels of 0·4–9·48 μg mL$^{-1}$ reported clinically in man (Lønning et al 1986; Harris et al 1986) (Table 1).

Table 1. Plasma levels of aminoglutethimide and % antinociceptive effect as seen in the abdominal constriction test in mice. AG was administered as a suspension in 0·1% w/v carboxymethylcellulose/0·1% v/v Tween 80, 0·1 mL/10 g weight, 45 min before challenge with the intraperitoneal irritant (3% v/v acetic acid), 0·1 mL/10 g. The % antinociceptive effect was calculated as described. Plasma levels of aminoglutethimide (AG) were determined 1 h after oral administration of the drug by reverse phase HPLC after dichloromethane extraction.

| Dose of AG mg kg$^{-1}$ | Plasma levels of AG μg mL$^{-1}$ | Antinociceptive effect (%) ± s.e.m. |
|---|---|---|
| 10 | 2.0 | 0·73 ± 0·43 |
| 20 | 5·5 | 21·3 ± 2·3 |
| 50 | 17·0 | 28·0 ± 1·6 |
| 100 | 36·6 | 32·9 ± 2·4 |

*The abdominal constriction test* showed AG to potentiate the antinociceptive activity of the NSAIDs ($P < 0·02$, Table 2). The interactions with the other drugs (dextromoramide, dihydrocodeine, morphine and paracetamol) were not significant ($P > 0·05$).

*The tail immersion test* showed only the 'centrally acting' agents were active (Table 3) and their protective effect was not enhanced by AG (itself inactive in this test). The indomethacin- and paracetamol-treated animals behaved no differently from the control animals ($P > 0·05$), as would be expected in this test.

## *Discussion*

The data presented show that AG significantly potentiates the antinociceptive effect of the NSAIDs in the abdominal constriction test. There are several possible explanations for this—one being that it is the result of an

Table 2. Antinociceptive effect of standard analgesics ± aminoglutethimide as seen in the abdominal constriction test in mice. All drugs were administered orally as a suspension in 0·1% w/v carboxymethylcellulose/0·1% v/v Tween 80, 0·1 mL/10 g weight, 45 min before challenge with the intraperitoneal irritant (3% v/v acetic acid, 0·1 mL/10 g). Antinociceptive effect was calculated as described. All s.e.m.s < 1·5, n = 16. Effective submaximal doses of the standard analgesics were used. Dose of aminoglutethimide (AG) 10 mg kg$^{-1}$. Antinociceptive effect of AG alone < 2%. Effect enhanced (*$P$ < 0·02) compared with the antinociceptive effect of analgesic given alone.

| Drug | Dose mg kg$^{-1}$ | Antinociceptive effect (%) −AG | +AG |
|---|---|---|---|
| NSAIDs | | | |
| Aspirin | 100 | 31 | 56·3* |
| Ibuprofen | 20 | 46 | 62 * |
| Indomethacin | 20 | 29·5 | 54·6* |
| Mefenamic Acid | 50 | 50·3 | 63·4* |
| Others | | | |
| Dextromoramide | 5 | 42 | 38 |
| Dihydrocodeine | 5 | 63 | 59 |
| Morphine | 2 | 39·5 | 41·6 |
| Paracetamol | 200 | 28·2 | 25·1 |

Table 3. Antinociceptive effect of analgesics ± aminoglutethimide in the mouse tail immersion test at 48 °C. All drugs were administered orally as a suspension in 0·1% w/v carboxymethylcellulose/0·1% v/v Tween 80, 0·1 mL/10 g weight. Dose of aminoglutethimide (AG) 10 mg kg$^{-1}$. n = 20.

| Drug | Dose mg kg$^{-1}$ | Antinociceptive effect (%, ±s.e.m.) −AG | +AG |
|---|---|---|---|
| Dihydrocodeine | 5 | 81·0 ± 14·9 | 77·8 ± 12·3 |
| Indomethacin | 20 | 4·5 ± 4·5 | 16·7 ± 7·9 |
| Morphine | 2 | 71·1 ± 6·6 | 63·8 ± 6·6 |
| Paracetamol | 200 | 17·4 ± 4·9 | 25·9 ± 5·2 |

* Expressed as a % vehicle-treated animals.

inhibitory effect of AG on drug metabolism. If, however, this were the case it might be expected that all the agents would be affected in a similar manner.

AG is a competitive inhibitor of several cytochrome P450 enzyme systems (Santen 1981; Daly et al 1986; Nicholls et al 1986). This action would affect the metabolism of agents eliminated by oxidation. However, the drugs we used are not metabolized to any great extent by this pathway, most being conjugated with either glycine (e.g. aspirin) or with glucuronic acid (e.g. morphine and paracetamol). Therefore inhibition of P450-dependent systems would not greatly affect the metabolism (and thus the pharmacological effect) of such analgesics.

An interaction at the drug metabolism level cannot be excluded for AG's interactions with some drugs. We have recently reported (Nicholls & Rao 1986) that the antinociceptive activity of meptazinol is potentiated by AG and that this interaction appears to be of a pharmacokinetic nature.

The tail immersion test is used mainly to identify agents such as the narcotic agonists and partial agonists (e.g. morphine, diamorphine, pentazocine)—the so-called 'centrally acting' analgesics. The peripherally actings agents (e.g. aspirin, indomethacin, paracetamol) are generally inactive (Sewell & Spencer 1976). AG was not active in this test although it had an apparently dose-related antinociceptive activity in the abdominal constriction test, and there potentiated only the effects of the NSAIDs. This lack of activity in the tail immersion test would be expected of a peripherally acting (NSAID-like) drug. AG has central effects, as in high doses it produces some sedation and these may be responsible for the protective effects seen in the abdominal constriction test. However, the dose of AG used (10 mg kg$^{-1}$) was not sedative and, even if it had been, some protection would have been expected in the tail immersion test since central nervous system depressant drugs may show activity in this test.

AG has been reported to lower prostaglandin metabolite levels in patients being treated for metastatic breast cancer (Harris et al 1983a), and also to inhibit adenosine diphosphate-induced platelet aggregation (Rao & Nicholls 1986). These, together with the evidence that both male and female patients with metastatic disease are helped by AG, provides some evidence that AG may have a NSAID-like effect and that more than suppression of steroid biosynthesis is involved in its mode of action.

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# Effect of pre-existing inflammation on carrageenan-induced paw oedema in rats

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Twenty-four hours after injection of carrageenan into one hind paw, injection of the same amount into the contralateral paw produced a significantly attenuated inflammatory response. However, when the second injection was given 7 days later, the inflammation induced in the contralateral paw was comparable with the initial response to carrageenan. A time-course study of carrageenan-induced inflammation in rats showed that significant oedema persisted 24 h after carrageenan administration and complete recovery was achieved in 7 days. The attenuated inflammatory response in the contralateral paw after 24 h was antagonized by bilateral adrenalectomy and chemical sympathectomy induced by 6-hydroxydopamine. Carrageenan-induced paw oedema was also significantly less in rats with subacute inflammation induced by the croton oil granuloma pouch technique. This attenuated response was antagonized by pretreatment of the rats with metyrapone, an inhibitor of adrenocorticoid synthesis, and by 6-hydroxydopamine. It is likely that the pre-existing acute or subacute inflammation attenuates the inflammatory response of carrageenan, by acting as a stressor, inducing activation of the sympatho-adrenal system.

Experimental stress has been reported to exert an attenuating effect on inflammation. Overcrowding- (Sofia 1980) and cold- (Glenn & Gray 1965) induced stress have been shown to inhibit adjuvant-induced inflammation, while surgical stress reduces the inflammatory response of phlogistic agents (Brown et al 1968). We have recently shown (Bhattacharya et al 1987) that immobilization-induced stress in rats induces a time-related reduction in the intensity of carrageenan oedema. A second injection of carrageenan into the contralateral paw after an interval of 2 h, has been reported to elicit faster hyperalgesia even though the oedematous response was much reduced (Ferreira et al 1978). It was suggested that the latter effect was due to the counter-irritant action of the first contralateral injection of the phlogistic agent. However, it is possible that the inflammation induced by the initial injection of carrageenan acts as the nocisponsive stressor to activate the sympatho-adrenal system, thereby attenuating subsequent inflammation. This possibility has now been investigated.

## *Materials and methods*

The study was conducted on male Wistar strain albino rats (120–180 g), housed individually at an ambient temperature of 25 ± 2 °C and 45–55% relative humidity, with a 12 h light–dark cycle. The rats were fed Hind Lever pellet chow with water freely available. The experiments were made between 0900 and 1400h, during the light phase.

Paw inflammation was induced by carrageenan (0·1 mL of 1% suspension in 0·9% saline) injected below the plantar aponeurosis of the left hind paw (Winter et al 1962). A second injection of carrageenan was given into the right hind paw of the same rats 24 h later. Paw volumes, up to the ankle joints were measured before and at hourly intervals for 4 h, and after 24 h, and in some instances 7 days, following carrageenan administration, by means of a mercury plethysmograph. The increase in the paw volume was expressed in units representing 1 cm (volume = 0·075 mL) length of the displaced mercury.

In another group of rats, subacute inflammation was induced by the croton oil (2% in arachis oil) granuloma pouch technique (Selye 1953). An air pouch was created by injecting 25 mL air into the loose connective tissue between the shoulder blades of the rat and 0·5 mL croton oil was then injected into the pouch. On the 3rd day the pouch was compressed manually to prevent adhesions. The volume of the inflammatory exudate within the pouch on the 6th day has been found to be 1·07 ± 0·11 mL (n = 21) in pilot experiments (Das 1983). Carrageenan inflammation was induced in this group in the left hind paw on the 6th day after croton oil injection, and the paw volume was recorded before and at hourly intervals for 4 h after carrageenan.