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COMMUNICATIONS

AG-3-5: a chemical producing sensations of cold

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Menthol (Fig. 1) is widely used because it produces sensations of coolness and because it has a pleasing mint flavour and odour (USDHEW Publication 1979). The cold sensations elicited by menthol are a fact of human experience. In animal studies, it has been shown that menthol exerts a selective stimulation of peripheral cold receptors in the tongue, as measured by action potentials in the lingual nerve of the cat (Hensel & Zotterman 1951). We describe here a chemical, AG-3-5, which, like menthol, has the unusual property of producing cold sensations.

AG-3-5 represents a class of compounds known as tetrahydropyrimidine-2-one derivatives (Fig. 1). In 1972, Podesva & DoNascimento of Delmar Chemicals, Ltd discovered that such compounds affect behaviour in animals. Processes for making these compounds were patented by these investigators. The effects of AG-3-5 were first described by Burford & Chappel (1972). Pharmacologists working at Delmar Chemicals, Ltd stated that AG-3-5 produced in rats and monkeys, among other effects, a behavioural change known as 'wet shakes' which are rotational body movements of animals similar to those made by a dog when wet. We were interested in AG-3-5 because we had been studying narcotic withdrawal and one prominent feature of withdrawal in rats is repetitive shaking movements of the body (Wei 1981).

A sample of AG-3-5 was obtained from Delmar Chemicals, Ltd and, when tested in rodents, the shaking behaviour was observed (Wei 1976). Low doses of AG-3-5, usually less than 1 mg kg^{-1}, were sufficient to produce a high frequency of shaking. For example, 1 mg kg^{-1} of AG-3-5 administered intraperitoneally to rats elicited over 300 shakes in a 2 h observation period. AG-3-5 was effective in all laboratory species examined, namely, mice, rats, hamsters, gerbils, guinea-pigs, rabbits, cats and dogs (Wei 1976).

The reasons for the pharmacological activity of AG-3-5 gradually became apparent as these experiments were conducted. In order to administer AG-3-5 to animals, this compound was dissolved in propylene glycol, a relatively non-toxic solvent frequently used in drug formulations. When the drug solution accidentally came into contact with our mucous membranes—nostrils, lips and eyelids—we noticed sensations of coolness which lasted for approximately 15 min. These sensations of coolness were also experienced by six other individuals in our laboratory who have had occasion to work with AG-3-5. Propylene glycol by itself was not active. On the basis of these preliminary observations, we decided to compare AG-3-5 to menthol, to examine its toxicity, and to taste and ingest a small quantity of the drug so that the sensations produced by this chemical might be characterized.

Experiments were conducted on male albino rats, 250–450 g. In confirmation of previous results (Wei 1976), AG-3-5, dissolved in propylene glycol 1 mg kg^{-1}, produced in rats numerous and vigorous 'wet shakes' within 2 min of intraperitoneal (i.p.) injection. The median effective dose (ED50) for shaking, with a positive response defined as an animal which shook more than 10 times in the 10 min interval after injection, was estimated (Weil 1952) to be 0·18 mg kg^{-1} (Table 1). Injections of menthol, dissolved 125 mg ml^{-1} in 50% v/v ethanol-water, also produced shaking, the ED50 being 35 mg kg^{-1} i.p. The duration of shaking observed with menthol was shorter than for AG-3-5 possibly because at menthol doses >125 mg kg^{-1}, animals became ataxic and lost their righting reflex. These high menthol doses also produced stretching movements of the abdomen (writhing) which may be indicative of local irritation.

To determine the short-term toxic effects of AG-3-5, doses of up to 1·5 g kg^{-1} of Ag-3-5 were administered either orally or injected i.p. Surprisingly, none of the animals tested at these doses died or showed signs of debilitation or body weight loss in the one-month observation period following drug administration. This low acute toxicity of AG-3-5 was also mentioned in the patent disclosure for AG-3-5 but no data were presented (Podesva & DoNascimento 1974). By contrast, we confirmed the value for the median lethal dose (LD50)

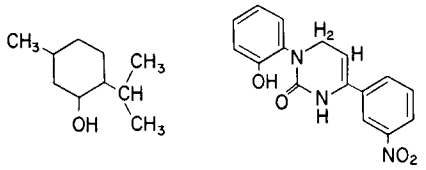

FIG. 1. Structures of menthol (2-isopropyl-5-methylcyclohexanol) and AG-3-5 (1-[2-hydroxyphenyl]-4-[3-nitrophenyl]-1,2,3,6-tetrahydropyrimidine-2-one).

* Correspondence.

Table 1. Comparison of the median lethal and effective doses of menthol and AG-3-5 in rats (mg kg^{-1} i.p.).

Chemical	LD50	ED50
(−)Menthol	700	35·0
AG-3-5	>1500	0·18

of menthol (Table 1), reported in the literature (FAO Report 1968). Deaths from high doses of menthol were due to the well-known anaesthetic properties of this compound as animals lost their righting reflex and respiration was diminished.

AG-3-5 was assayed in the Ames *Salmonella typhimurium* system, a short-term test which detects mutagenic chemicals that react via electrophilic intermediates (Ames et al 1975). This test was conducted because AG-3-5 has a nitrobenzene group in its molecular structure (Fig. 1) and it is known that the nitro group on the aromatic rings of many chemicals can be converted by tissue nitroreductases to toxic electrophilic intermediates (Chiu et al 1978; Poirier & Weisburger 1974). Positive results for mutagenicity were obtained for AG-3-5 in strain TA100 in the presence of a mammalian liver enzyme preparation (+S9). A linear increase in the number of revertant colonies was obtained in the range of 0·1–0·8 mg/plate using standard procedures (Ames et al 1975). The mutagenic potency was low; 105 net TA100 revertants were produced per 0·5 mg of AG-3-5, and 0·7 mg/plate was required to produce a doubling of the background number of revertants. At higher doses, >1 mg/plate, AG-3-5 precipitates appeared in the agar and no further increases in the number of revertant colonies were obtained. For comparison, our laboratory values for the positive control, 2-aminofluorene, were 90 TA100 revertants per microgram (+S9).

The low mutagenic potency of AG-3-5 suggests that chemical impurities in the AG-3-5 sample may account for its mutagenic activity. Such impurities are frequently encountered in the synthesis of nitro compounds (Donahue et al 1978; Jin et al 1982), and could also occur in the synthesis of AG-3-5 (Podesva & DoNascimento 1974). One of us (E.W.) decided on the basis of the Ames test data and the toxicity studies in rats that it was relatively safe to ingest a small amount of AG-3-5 in order to assess its pharmacological activity.

A micropipette was used to apply 0·1 mg of AG-3-5, dissolved 10 mg ml^{-1} in propylene glycol, onto the dorsal surface of the tongue. Prickling sensations of cold were produced by AG-3-5, similar to those of menthol but different in that discrete spots of cold could be felt. These effects lasted for 15 to 30 min and were not obtained with the drug vehicle, propylene glycol. In three separate experiments, 5 to 10 mg of AG-3-5 were ingested. AG-3-5, in powder form, or dissolved in propylene glycol or dimethyl sulfoxide, was mixed with a glass of orange juice and swallowed. On all 3 occasions AG-3-5 produced sensations of coldness in the mouth, pharynx, and in the chest down to the xyphoid process. Mild sensations of coolness were also experienced on the cheeks and on the inner surfaces of the arms and legs. These latter effects were, however, obtained in only one experiment. The cold sensations lasted from 30–60 min and were pleasant.

To determine if AG-3-5 was effective from the dermal route, it was applied, 5 mg ml^{-1} in propylene glycol or 50 mg ml^{-1} in dimethyl sulfoxide, to the dorsal surface of the forearm. No local or systemic sensations of cold were produced although the garlic odour of dimethyl sulfoxide could be detected in the breath. These experiments suggest that AG-3-5, a relatively insoluble compound in water but soluble in polar solvents, does not readily penetrate the intact skin surface to reach cold receptors in the epidermal basement membrane (Hensel 1973).

These results show that AG-3-5, like menthol, can selectively act on peripheral cold receptors in the upper alimentary tract to produce sensations of cold in man. Perhaps, in animals, intense activation of peripheral cold receptors after systemic administration of these drugs produces the response called 'wet shakes'. The functional utility of the shake response in animals may be related to attempts to remove cutaneous irritation (Sherrington & Laslett 1903; Sherrington 1917) and may also be a form of thermogenesis.

AG-3-5 is more potent than menthol and, because it lacks anaesthetic properties, has a lower acute toxicity. AG-3-5 also lacks the flavour and odour of menthol and is not readily absorbed through the skin. These properties suggest some potential pharmacological applications. For example, AG-3-5 may be useful in counteracting the pain of heat or burns or the hot flushes of menopause. The potential uses of AG-3-5 may be less restrictive than menthol because the flavour and odour of menthol, at high doses, can become unpalatable. The recognition that chemicals such as AG-3-5 can produce, as their principal action, sensations of cold, may also lead to an improved understanding of the chemical basis of temperature perception.

We propose that AG-3-5 be given the name 'icilin' because of its cold producing properties.

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A reappraisal of the equations used to predict the internal stresses in film coatings applied to tablet substrates

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It is now generally accepted that when a polymer film coating is applied to a substrate an internal stress (P) is invariably developed. This is composed of the sum of the internal stress due to shrinkage of the film on evaporation of the solvent (P_S), and the thermal stress due to differences in the thermal expansion of the film coating and substrate during changes in temperature arising out of the coating process (P_T). Recently Croll 1979) and Sato (1980) presented equations for the calculation of P_S and P_T respectively in organic coatings:

$$P_S = \frac{E}{1-\nu}\left[\frac{\phi_s - \phi_r}{3(1-\phi_r)}\right] \quad (1)$$

$$P_T = \frac{E}{1-\nu^2}\,\Delta\alpha\Delta T \quad (2)$$

Where E is the Young's modulus of elasticity of the coating; ν is the Poisson's ratio of the coating; ϕ_s is the volume fraction of the solvent at the solidification point (i.e. where the coating solution first behaves as a solid rather than a viscous liquid); ϕ_r is the volume fraction of the solvent remaining in the dry coating at ambient conditions; $\Delta\alpha$ is the difference between the coefficient of linear expansion of the coating, α_c, and the substrate, α_s; ΔT is the difference between the glass transition temperature of the coating, T_g, and the ambient temperature, T. Unfortunately, Sato (1980) did not report any derivation of his equation and hence the assumptions made can only be a matter for conjecture. However, a detailed study of several other treatments (Chow et al 1976; Croll 1978, 1979; Hoffman 1981) has revealed that equation (2) may not be totally applicable to tablet film coatings.

As usual in all problems of elasticity (in all cases the coating is regarded as a Hookean solid) the strains and corresponding stresses are connected by means of the elastic constants. The strains in the two cases are different in origin; Croll (1979) assumed the linear shrinkage strain at the interface to be one third of the internal bulk strain within the coating due to volume of solvent lost from the film after solidification, while Sato (1980) assumed the differential thermal strain to be equivalent to the difference in the linear expansion of both the coating and substrate over the temperature range $T_g - T$. Both assumptions are valid in the context of tablet film coatings. Since both processes give rise to what is known as a plane stress situation, i.e. the coating has no stress normal to its plane, all stresses lying within the plane of the coating, then the relevant equation relating the induced stress in an arbitrary direction, σ, to the strains is given by:

$$\sigma = \frac{E}{1-\nu^2}(\varepsilon + \nu\varepsilon_1) \quad (3)$$

where ε and ε_1 are the strains parallel and perpendicular to the stress.

It can be seen that if $\varepsilon_1 = \varepsilon$ then:

$$\sigma = \frac{E}{1-\nu}\,\varepsilon \quad (4)$$

but if ε_1 is zero then:

$$\sigma = \frac{E}{1-\nu^2}\,\varepsilon \quad (5)$$

Sato (1980) appears to have considered the latter case as used by Chow et al (1976) in defining the stress at the film/substrate interface along a coated cantilever beam. It does not take into account shrinkage of the coating across the cantilever resulting in an equal strain perpendicular to its length. A consideration of this more general situation results in equation (4) as used by Croll (1978, 1979) and Hoffman (1981). For tablet film