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SK&F 93574, a histamine H_2-receptor antagonist, releases histamine in the dog

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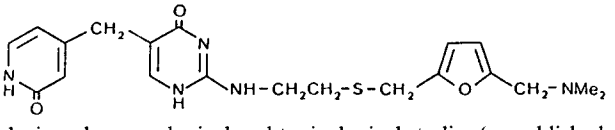
Abstract—This study was designed to establish whether SK&F 93574 releases histamine in dogs. Three female beagle dogs each received single infusions (on separate days) of each of SK&F 93574 (2·5 mg kg^{-1}), polyvinylpyrrolidone (PVP, 20 mg kg^{-1}) and sterile saline. The treatments were given at 14 day intervals by rapid intravenous infusion at 0·5 mL kg^{-1} min^{-1} for 2 minutes. Dogs showed clinical signs of histamine release such as vasodilation, licking lips, head drooping and increased gut movement after treatment with the known histamine releaser PVP or the test compound SK&F 93574. These signs were of similar severity and duration for the two compounds. No such changes were observed when the dogs received vehicle alone. Treatment with PVP or SK&F 93574 also resulted in markedly elevated plasma histamine concentrations (> 10-fold increase over control). It is concluded that intravenous administration of SK&F 93574 to dogs is associated with histamine release.

SK&F 93574 (I) is a potent and long acting histamine H_2-receptor antagonist (Blakemore et al 1985). It was observed



during pharmacological and toxicological studies (unpublished data) that some dogs treated with SK&F 93574 collapsed and showed clinical signs which were consistent with histamine release.

A wide variety of compounds is known to release histamine (Paton 1957). Xenobiotics and endogenous substances containing one or more amine group are thought to act partly by displacing histamine from its binding sites and partly by facilitating exocytotic release of the storage granules (see Bowman & Rand 1980). It seems that the histamine H_1-receptor antagonists may act directly in this way. The effect of H_2-receptor antagonists (blockade of histamine-induced inhibition of histamine release) may be related to the existence of histamine H_2-receptors on basophils (Lichtenstein & Gillespie 1975). Large molecules such as compound 48/80, dextran and polyvinylpyrrolidone are also able to release histamine (see Paton 1957).

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SK&F 93574 is a highly basic drug with several amine groups and consequently may have the potential for this effect. The present study was designed to establish whether, in beagle dogs, this was the case.

Methods

Animals. Purebred beagle dogs between 2 and 4 years old, 10–16 kg, were housed singly in pens and allowed access to SDS dog diet (Special Diet Services Ltd., Witham, Essex, UK) for 1 h a day and free access to drinking water.

Clinical procedure. The dogs each received single infusions of each of saline, SK&F 93574 (2·5 mg kg^{-1}) and positive control compound PVP (20 mg kg^{-1}). The treatments were given in a 'Latin square'-type order at 14 day intervals. Preliminary experiments (not reported) had shown that if a second dose of a histamine releaser was given 7 days after a single dose then a diminished response was seen after the second dose, but at a 14 day interval the responses were of the same magnitude.

The infusions were made with the dogs conscious but restrained in a Pavlov sling. Indwelling cannulae (21 gauge intravenous infusion set, Portex, Hythe, Kent) were placed into both cephalic veins 30 min before treatment. Drug was infused via the cannula in the right cephalic vein. Ten minutes after the infusion (or earlier if there were marked clinical changes) a blood sample was withdrawn from the cannula in the left cephalic vein. Clinical observations were recorded during and after each treatment.

To accustom the dogs to the procedure, and so minimize the effects of stress (which may alter histamine concentrations), sham experiments were carried out on two occasions (3 weeks and 2 weeks) before the first treatment day. On these occasions the cephalic veins were cannulated, 2 mL of saline was injected, and a blood sample was obtained.

Preparation of plasma. The blood samples were taken into heparinized syringes (final concentration approx 15 iu heparin per mL blood), immediately centrifuged (1600*g*, 4°C), and the plasma stored below −40°C until assayed for histamine. The blood was handled gently to minimize disruption of basophils.

Several millimetres of plasma immediately above the red cells were left untouched, again to avoid disrupting the basophils.

Plasma aliquots for HPLC assay were deproteinized with perchloric acid (1 volume 2 M perchloric acid to 3 volumes plasma) and centrifuged again before storage of the supernatant at −40°C.

Radioimmunoassay for histamine. Plasma histamine concentration were estimated using a histamine radioimmunoassay kit obtained from NMS Pharmaceuticals Inc., Newport Beach, California, USA.

HPLC assay for histamine. Deproteinized plasma was adjusted to pH 7·8, extracted on a carboxylic acid column (Bond Elut, Analytichem International, Harbor City, California, USA) and the histamine eluted off in a 0·1 M HCl. This was adjusted to pH 9, reacted with *o*-phthaldialdehyde (Fluoraldehyde Reagent, Pierce & Warriner, Chester, UK) adjusted to pH 6 and 20 μL immediately injected onto the HPLC system.

Separation was carried out on Beckman Ultrasphere Octyl columns (45 mm × 4·6 mm × 5 μm followed by 150 mm × 4·6 mm × 5 μm) at ambient temperature. The solvent was 54% methanol, 46% 0·01 M sodium phosphate pH 6 and the flow rate was 0·7 mL min^{-1}. The column effluent was passed into a Perkin Elmer LS5 fluorescence spectrometer (excitation 340 mm, emission 440 mm) which was connected to a Spectraphysics SP4290 integrator.

Results

Clinical observations. No adverse signs were seen during or after treatment with saline except, on one occasion, slight licking of the lips was seen (Table 1). The signs seen after treatment with SK&F 93574 or PVP were virtually identical in severity and duration. Characteristically, these were vasodilation, licking lips, head droop or collapse, and often watery eyes or nose, defaecation and urination. The onset of these effects was during the first minutes after the start of this infusion. The animals completely recovered within 2–3 h.

Table 1. Clinical observations.

Dog no.	Responses to treatment		
	Saline	SK&F 93574	PVP
1	—	Vasodilation Watery eyes and nose Licking lips Defaecation Head droop	Vasodilation Licking lips Defaecation, urination Collapsed Panting Agitation, aggression
2	(Licking lips)	Vasodilation Watery eyes Licking lips Flatulence, defaecation Head droop	Vasodilation Licking lips Head droop, collapse Panting Agitation Frowning
3	—	Vasodilation Watery eyes and nose Licking lips Defaecation, urination Head droop, collapsed Panting	Vasodilation Nasal discharge Licking lips, salivation Defaecation, urination Head droop, unsteady Panting Shaking head, yawning Feet shuffling

Radioimmunoassay for plasma histamine. The sensitivity of this assay was approx 1 ng mL^{-1} and the within assay coefficient of variation was 17% at a mean concentration of 16·1 ng mL^{-1} and 10·6% at a mean concentration of 5·8 ng mL^{-1}. There was no difference in the plasma histamine concentrations measured on either the pretest days or the saline treatment day (13·2–18·2 ng mL^{-1}; Table 2). In all three dogs treatment with SK&F 93574 or PVP resulted in markedly elevated histamine concentrations (> 200 ng mL^{-1}).

Table 2. Plasma histamine concentrations (ng mL^{-1}).

Dog no.	Pretest		Treatment		
	1	2	Saline	SK&F 93574	PVP
1	16·4	15·2	13·2	200	300
2	16·8	18·2	18·2	220	260
3	15·2	—	15·2	>400	370

(— = not measured).

HPLC assay for histamine. This was undertaken to provide qualitative evidence of circulating histamine in some dogs in order to support the radioimmunoassay results. The histamine peak had a retention time of approximately 12 min. It can be seen from Fig. 1 that there was no detectable histamine in the plasma from a saline-treated dog, but treatment with PVP or SK&F 93574 resulted in high plasma concentrations. Although, quantitatively this method proved to be less sensitive than the RIA method, it did provide confirmatory evidence that histamine release occurred after treatment with PVP or SK&F 93574.

Discussion

The clinical observations and plasma histamine concentrations show that SK&F 93574 stimulates the release of histamine when given by rapid intravenous infusion to dogs. Symptoms such as vasodilation, watering eye and nose, salivation and collapse, well known indicators of histamine release seen after administration of a variety of basic compounds, were observed following treatment with PVP or SK&F 93574.

No evidence exists at present to show whether SK&F 93574 causes direct release of histamine, or whether it has an indirect effect via the release of other substances. The mechanism appears to involve depletion of histamine stores as preliminary experiments (not reported) demonstrated that after 7 days repeated challenge with a known histamine releaser produced a diminished response that was not seen at 14 days (when stores had presumably been replenished).

The pretest and saline-treated plasma histamine concentrations measured (13·2 to 18·2 ng mL^{-1}) are much higher than some previously reported basal concentrations (up to 1 ng mL^{-1}; Lorenz et al 1974). However, they are of the same order as that reported by Halpern et al (1955; approx 10 ng mL^{-1}). This discrepancy may, at least in part, be due to the different methods used to measure histamine, or could be due to cross-reactivity with another endogenous substance. The major cross-reactivities of the antiserum are 6·2% with l-methylhistamine, 0·4% with spermidine and 0·02% with 5-hydroxytryptamine. The HPLC data show that treatment with PVP or SK&F 93574 resulted in an increase in the plasma concentration of a substance that co-eluted with histamine. Therefore, although we are unable to explain the high level basal concentrations measured, we are confident that treatment with PVP or SK&F 93574 did markedly increase plasma histamine concentrations. The increase we

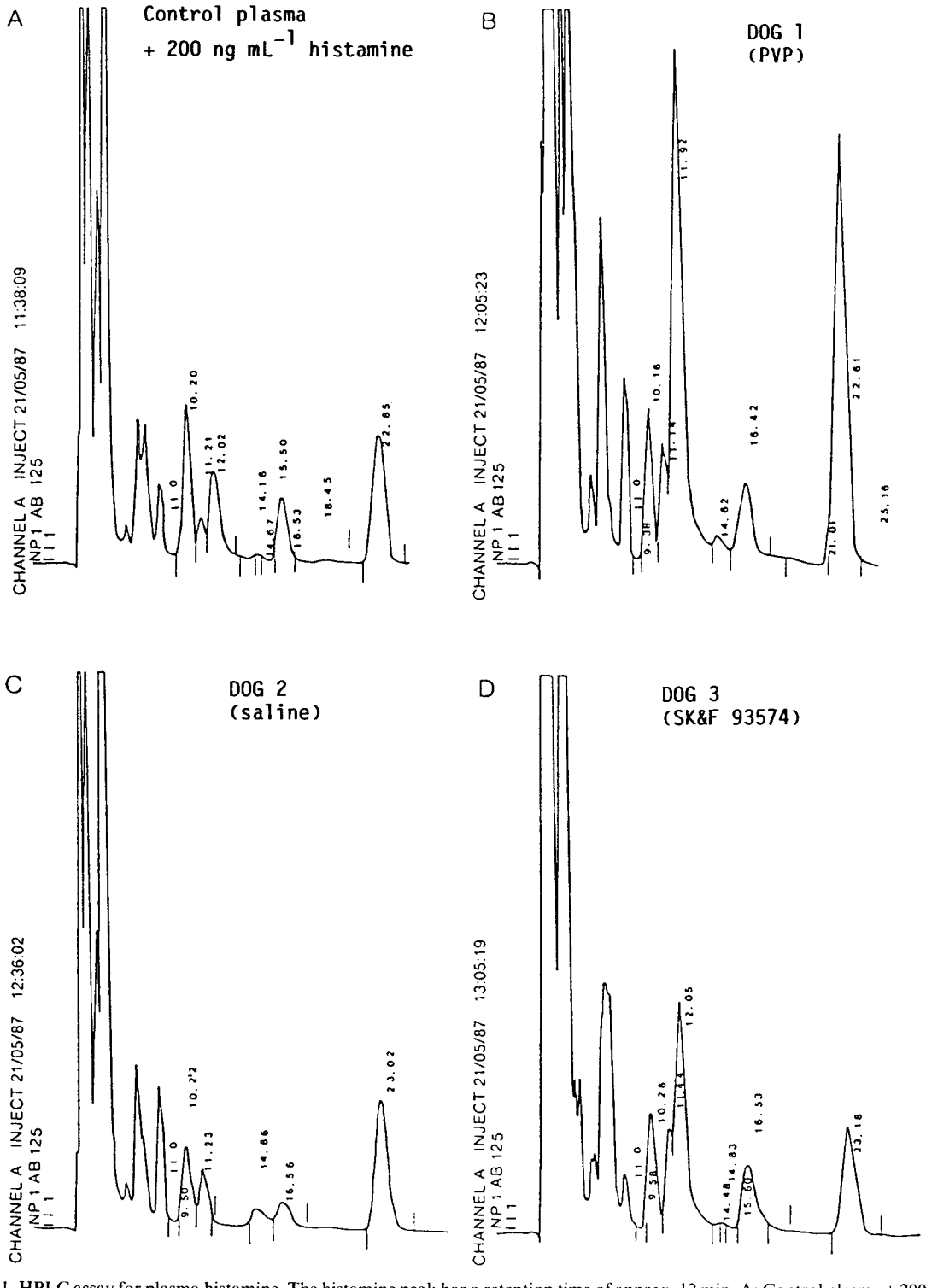

FIG. 1. HPLC assay for plasma histamine. The histamine peak has a retention time of approx. 12 min. A: Control plasma + 200 ng mL^{-1} histamine. B: Dog 1, treated with PVP, C: Dog 2, treated with saline. D: Dog 3, treated with SK&F 93574.

observed was of similar magnitude to that observed by Halpern et al (1955) who reported that PVP (62·5 mg kg^{-1}) produced an increase in plasma histamine concentration from approx 10 to approx 300 ng mL^{-1}.

To summarize, intravenous infusion of SK&F 93574 (2·5 mg kg^{-1} over 2 min) into dogs is associated with histamine release. The exact mechanism of release and any association with histamine receptors remains to be investigated.

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Letter to the Editor

Muscarinic receptor subtypes involved in bethanechol-induced water intake in the rat

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We have recently described a compulsive drinking behaviour elicited by bethanechol through activation of central muscarinic receptors in the rat (Schiavone et al 1987). The bethanechol-induced water intake was specifically inhibited by some antimuscarinic drugs, while being insensitive to histaminergic (H_1 and H_2), adrenergic (α and β) and 5-hydroxytryptaminergic antagonists.

The present report attempts to characterize the drinking response to bethanechol in relation to the muscarinic receptor subtype involved.

The method employed was that of Schiavone et al (1987). Briefly, water intake was measured for 30 min following injection of bethanechol, 10 μg/rat, into the right cerebral ventricle through a chronically implanted cannula. The effect of antagonists was evaluated after their intraventricular administration, 2 min before bethanechol challenge.

We used pirenzepine and AF-DX 116 as muscarinic antagonists selective for M_1 and M_2 (cardiac) receptors, respectively. Those compounds have provided the biochemical and functional basis for the differentiation of muscarinic receptors into subtypes (see reviews by Hirshowitz et al 1984; Levine et al 1988).

Results showed that both compounds were able to inhibit bethanechol-induced water intake, albeit with different potencies. The ID50 (and 95% confidence limits), expressed in pmol/rat, were 110 (18–700) and 1800 (610–5400) for pirenzepine and AF-DX 116, respectively.

Thus, when compared with the ID50 of the non-selective drug atropine, 6·7 pmol/rat (Schiavone et al 1987), the potencies of pirenzepine and AF-DX 116 were 17- and 270-fold lower, respectively. The potency of AF-DX 116 in some functional responses subserved by the cardiac muscarinic receptor, i.e. the subtype recognized as having high affinity for the drug, has been shown to be about one order of magnitude lower than that of atropine (Giachetti et al 1986; Micheletti et al 1987). The potency ratio found in the present study (270) falls in the range of magnitude (two to three orders) typical of effects due to receptors recognized by AF-DX 116 as having low affinity (Micheletti et al 1987). On this evidence, that the receptor activated by bethanechol belongs to the M_2 (cardiac) subtype can be confidently excluded.

Conversely, pirenzepine was about 1/20th as potent as atropine in inhibiting compulsive drinking. Despite the noticeable potency of pirenzepine, the receptor stimulated by bethanechol cannot be unambigously defined as M_1 for two main reasons: i) the lower lipophilicity of pirenzepine in comparison to atropine might increase the apparent efficacy of the former drug by slowing its diffusion rate from the active compartment. In this context it is worth mentioning that the order of lipophilicity is: AF-DX 116 > atropine > pirenzepine (log P at pH 12–14 being +2·2, +1·5, +0·1, respectively; Engel, personal communication); ii) cloning and sequencing of muscarinic receptor genes from pig brain (Kubo et al 1986a) and heart (Kubo et al 1986b; Peralta et al 1987) and rat brain (Bonner et al 1987) has demonstrated the existence of four separate receptor molecules. Three of these functional genes are expressed in the rat cerebral cortex, and are not clearly distinguished by pirenzepine, which binds them with homogeneous high affinity (Bonner et al 1987). It is therefore conceivable that anyone of the three subtypes is responsible for the bethanechol effect.

Recently, Hagan et al (1987) speculated that cholinergic stimulation of drinking from the lateral hypothalamus was mediated by M_2 receptors. This conclusion was based on the inefficacy of putatively selective M_1 agonists (McN-A-343, AHR 602, AH 6405) in promoting drinking, and on the lack of a systematic relationship between antagonist potency and their affinity for the M_1 receptor. Since McN-A-343 and related compounds do not discriminate among muscarinic subtypes as regards affinity, their lack of efficacy as drinking-inducers may depend on a small receptor number, presumably inadequate for partial agonists (Eglen & Whiting 1986). Interestingly, M_1 receptors represent only a small proportion of the total muscarinic receptors (Cortes & Palacios 1986) in brain regions from which a drinking response was elicited by direct intracranial injection of carbachol in the rat (Swanson & Sharpe 1973).

In conclusion, the receptor subtype responsible for muscarinic-induced water intake in the rat cannot presently be clearly identified other than as non M_2 (cardiac). The advent of more selective agents should help to clarify this issue.

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