amount usually drunk had no further selective effect on morphine consumption (trial blocks 4–6), although total fluid intake was reduced by 16% ($P = < 0.05$), indicating a general depressant effect of the high dose of injected morphine.

Pretreatment with nalorphine[5] and naloxone[6] depresses responding for intravenous doses of morphine in rats, and naloxone 'blockade' can reduce preferences for solutions of etonitazine[7]. In our experiment, pretreatment with naloxone (up to 8 mg per kg) produced neither an initial increase in morphine consumption[8] nor an eventual extinction of preferences. An even larger dose of naloxone, or injections of naltrexone, which is an antagonist with a longer action, might have been more effective, but other measures showed that both naloxone and morphine in the chosen dose ranges were exerting marked effects. The controls grew steadily and gained about 20 g over the 24 d of injections, whereas both the drugged groups failed to gain weight during the first 12 d of constant dosage. Increasing the dose of morphine to 120 mg per kg per d led to more weight loss, but, paradoxically, the rats given more naloxone began to gain weight; however, they remained extremely difficult to handle and had copious diarrhoea.

An aversion procedure was tested in 10 dependent rats (Fig. 2). Rats can develop long-lasting aversions to novel tastes if such tastes are associated with 'sickness'[9], and conditioned aversions to novel flavours paired with the morphine abstinence state[10,11] have been demonstrated. The morphine-drinking rats were therefore given their usual choice trials, but every third day they were instead given 0.5 h access to a solution with a new taste. This novel solution was the usual morphine 0.5 mg ml^{-1}, but with added saccharin sodium (0.5 mg ml^{-1}). Immediately after access to the morphine plus saccharin solution, the rats were injected with naloxone (1 mg per kg). Thus, the novel taste was paired on four occasions with precipitated abstinence from morphine. During intervening pairs of days, the rats continued with choice trials between morphine solution and water (choice trials pairs 1–4, Fig. 2). No further aversion training was given, and then, for the next 12 d, the rats were given either a choice between morphine and water as before, or, on alternate days, a choice between morphine plus saccharin and water. They clearly rejected the mixture, while maintaining self-administration of morphine ($t = 8.67$, $P < 0.005$). During the next 12 d (choice trial pairs 11–16), these rats continued to reject the solution of morphine plus saccharin, even though the consequence was 'self-imposed' morphine abstinence. Choice trials pairs 17–22 show that although saccharin alone was initially rejected, possibly reflecting enhanced neophobia[12], the rats very soon began to consume it in increasing amounts while still rejecting the mixture. The fifth phase (choice trial pairs 23–28) demonstrated that even after 24 d of 'voluntary' abstinence, the rats readily relapsed to morphine drinking when given morphine–water choice trials again. The slight reduction in the proportional consumption of morphine was not significant ($t = 1.14$). Tests in other groups of rats that are familiar with the taste of morphine, have confirmed that solutions of morphine plus saccharin are not rejected spontaneously[13].

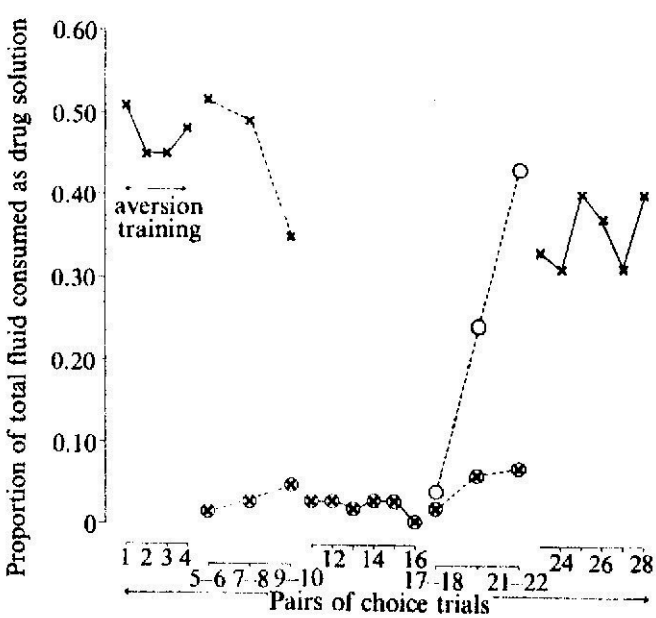

Fig. 2 An aversion to a novel flavour (morphine plus saccharin, ⊗) can be induced in rats which have learned to prefer morphine solutions (×) to water. This aversion is long lasting (trial pairs 5–22), and dependent rats abstain from morphine if the solutions to which they have been averted are made the only source of the drug. Giving choice tests on alternate days between morphine and water or morphine plus saccharin and water (trial pairs 5–10) shows that the aversion is selective, since the rats continue to ingest plain morphine with which they are familiar. Similar comparisons with saccharin alone (○) (trial pairs 17–22) show that this novel taste is initially rejected, but then the rats drink saccharin in rapidly increasing amounts while continuing to reject morphine plus saccharin. In spite of prolonged abstinence from morphine, the animals relapse when offered plain morphine solutions again (trial pairs 23–28).

In contrast with other studies[5,8,14], these experiments provide minimal evidence of regulation of opioid intake by dependent subjects and they emphasise the role of conditioned reinforcers[5,15,16] in sustaining morphine-seeking behaviour. The high relapse rate of the averted (24-d abstinent) rats also stresses the importance of learned tendencies. The ability of the averted rats to make subtle discriminations between tastes that might or might not be 'safe' underlines a major limitation of behavioural treatment techniques, which is a lack of generalisation of learning to stimuli outside the treatment setting.

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Serotonergic component of neuroleptic receptors

Binding studies performed *in vitro* with ^{3}H-haloperidol[1–3], ^{3}H-spiperone[4–6], ^{3}H-dopamine[1–3,6] and ^{3}H-apomorphine[7,8] showed that the specific neuroleptic binding sites in the striatum are dopaminergic. The frontal cortex, however, was much more labelled by ^{3}H-spiperone than by ^{3}H-haloperidol[9,10]. The investigations reported here indicate that the neuroleptic receptor sites in the frontal cortex labelled by ^{3}H-spiperone are predominantly serotonergic and virtually identical to those labelled by ^{3}H-LSD. These conclusions were reached from a study of the relative binding affinities of a series of serotonin or dopamine agonists or antagonists for rat frontal cortex and striatal receptors. Significant correlations were obtained between the binding activities in the rat frontal cortex and *in vivo*

potencies in the pharmacological anti-tryptamine test. Similarly, binding activities in the striatum were significantly correlated with the potencies in the anti-apomorphine test. We therefore suggest that serotonergic, as well as dopaminergic, receptors are involved in the mechanism of action of neuroleptic drugs.

The methods used to assay *in vitro* and *in vivo* receptor binding were essentially similar to those described previously[4,10,11]. *In vitro* experiments were carried out with a total particulate tissue preparation of rat striatum or frontal cortex. Incubation conditions were the same in all experiments: ^{3}H-ligand concentration of 2 nM; 11.4 mg tissue ml^{-1}; NaCl, 120 mM; KCl 5 mM; $CaCl_2$ 2 mM; $MgCl_2$, 1 mM; pargyline 10 μM; ascorbic acid 0.1% in Tris–HCl buffer 0.05 M, *p*H 7.6; volume 2.2 ml, 37 °C. After 10 min incubation was stopped by rapid filtration under suction through Whatman GF/B glass fibre filters. These were washed twice with 5 ml ice-cold buffer and then counted for radioactivity. Stereospecific binding of ^{3}H-spiperone and ^{3}H-haloperidol was taken as the difference between radioactivity on the filter in assays in the presence of (−) and (+)-butaclamol. When present in excess (10^{-5} M) the pharmacologically active (+)-enantiomer completely displaced the labelled ligand from its specific binding sites, whereas the inactive (−)-enantiomer had no effect. Blank values for ^{3}H-LSD binding were measured in the presence of excess cold LSD (10^{-6} M). Inhibition of specific binding by drugs was measured at 5 different concentrations and expressed as IC_{50} values (that is, drug concentration causing 50% inhibition of the specific binding of the labelled ligand). Male Wistar rats weighing 240±20 g were used for the *in vivo* pharmacological experiments. A detailed description of the methods has been reported previously[12], but briefly, rats were pretreated with the drugs and then injected intravenously with apomorphine–HCl (1.25 mg kg^{-1}) 0.5 h later. Stereotypy was scored every 5 min over a 1-h period. Thereafter, the animals were injected i.v. with 40 mg kg^{-1} tryptamine and bilateral clonic seizures of the forepaws were assessed immediately after injection. Based on control results, 'all or none' criteria were selected and used for the calculation of the ED_{50} values in μmol kg^{-1}. Multiple regression analysis was carried out using a AQSARF (automatic quantitative structure activity finder) program[13].

In the rat frontal cortex *in vitro* stereospecific binding of ^{3}H-spiperone amounted to 23 pmol per g tissue. This represented 70% of the total binding and half the amount of stereospecific binding in the striatum. ^{3}H-LSD also showed a high specific binding in the frontal cortex (9.8 pmol per g tissue). For ^{3}H-haloperidol, however, the binding in the frontal cortex was ten times lower than in the striatum[9].

Specific *in vitro* binding in the frontal cortex of both ^{3}H-spiperone and ^{3}H-LSD was inhibited more by serotonin-like compounds than by dopamine agonists (Table 1). The reverse was true for *in vitro* binding of ^{3}H-haloperidol and ^{3}H-spiperone in the striatum. Serotonin antagonists were more potent inhibitors in binding tests in the frontal cortex than in the striatum (Table 1). Comparison of the binding results with the results of other *in vivo* pharmacological tests (Table 1) shows that binding in the frontal cortex is correlated with the anti-tryptamine activity of the drugs. From Table 1 it can also be seen that the order of compounds measured by the ratio of their *in vitro* binding activity (IC_{50} in ^{3}H-haloperidol binding in the striatum to the IC_{50} of ^{3}H-spiperone binding in the frontal cortex) closely parallels that obtained from the ratio of their *in vivo* pharmacological activity (ED_{50} in anti-tryptamine test to the ED_{50} in the anti-apomorphine test).

Table 2 shows that in the series of neuroleptics of different chemical classes the IC_{50}-values of *in vitro* binding in the frontal cortex and in the striatum varied widely. Regression analysis of the IC_{50} values of the *in vitro* binding tests revealed a highly significant correlation between the values obtained for ^{3}H-spiperone and ^{3}H-LSD binding in the frontal cortex (correlation coefficient $r = 0.93$) and also between the values for ^{3}H-spiperone and ^{3}H-haloperidol binding in the striatum ($r = 0.92$). A linear correlation was not obtained between the IC_{50} values of two different brain areas. Certain compounds were more active in the frontal cortex binding test than in the striatal one. Pipamperone showed the highest dissociation (ratio of *in vitro* IC_{50} values = 20), having a ratio even higher than the serotonin agonists and most of the serotonin antagonists. On the other hand, the benzamides were 100 to 1,000 times more active in the striatal binding tests but were only weakly active overall. Specific and potent dopamine antagonists, like

Table 1 *In vitro* potency of (*a*) amine agonists and (*b*) 5-hydroxytryptamine (serotonin) antagonists in binding tests using rat striatum and frontal cortex and *in vivo* antagonism of apomorphine-induced stereotypy and tryptamine-induced clonic seizures in rats

	Inhibition of specific binding −log IC_{50} (M)±s.e.m.*				Antagonism *in vivo* ED_{50} (μmol kg^{-1})		Ratio	
	Striatum		Frontal cortex		Apomorphine	Tryptamine	*In vitro*†	*In vivo*‡
	^{3}H-spiperone	^{3}H-haloperidol	^{3}H-spip.	^{3}H-LSD	stereotypy	seizures	activities	activities
a								
Bufotenin	4.40±0.05	4.7±0.1	5.8±0.1	6.20±0.07			13	
5-Hydroxytryptamine	4.10±0.05	4.5±0.1	5.5±0.1	6.1±0.1			10	
LSD	7.25±0.05	7.3±0.1	7.6±0.1	8.3±0.1			2	
Tryptamine	4.05±0.05	4.6±0.1	4.78±0.07	5.3±0.1			1.5	
Apomorphine	6.3±0.1	7.3±0.1	5.0±0.1	5.5±0.1			0.005	
Dopamine	5.2±0.1	6.0±0.1	3.6±0.1	3.7±0.1			0.004	
2-(*N*, *N*-dipropyl) amino-5, 6-dihydroxy tetralin	6.9±0.2	7.8±0.1	4.1±0.1	4.4±0.1			0.0002	
b								
Mianserine	5.7±0.1	5.8±0.1	7.4±0.1	7.35±0.05	266	2	40	133
Cinanserine	5.0±0.1	5.4±0.1	6.90±0.05	6.95±0.05	452	9.5	32	48
Methysergide	6.5±0.1	6.3±0.1	7.45±0.05	8.10±0.05	120	1.5	14	80
Pizotifen	6.3±0.2	6.6±0.1	7.7±0.1	7.7±0.1	102	0.9	13	113
Cyproheptadine	6.7±0.1	7.1±0.1	7.7±0.1	7.55±0.05	65	1	4	65

*Mean values were calculated from three independent determinations using freshly prepared tissue and drug solutions, and performed in duplicate.

Presently shown IC_{50} values were not converted into K_i values using the Cheng–Prusov equation, since both receptor populations in striatum and frontal cortex seemed to be heterogeneous and the real dissociation constant of the labelled ligand cannot be measured.

†Ratio of *in vitro* activities is calculated as IC_{50} (halo., striatum)/IC_{50} (spip., frontal cortex), expressing within the series for the various compounds differences in dissociation between affinity for frontal cortical and striatal receptors.

‡Ratio of *in vivo* activities is calculated as ED_{50} in apomorphine test to ED_{50} in tryptamine test.

Table 2 *In vitro* potency of 23 neuroleptics in binding tests using rat striatum and frontal cortex and *in vivo* antagonism of apomorphine-induced stereotypy and tryptamine-induced clonic seizures in rats

	Inhibition of specific binding $-\log IC_{50}$ (M)±s.e.m.*				Antagonism *in vivo* ED_{50} (μmol kg^{-1})		Ratio	
	Striatum		Frontal cortex					
	^{3}H-spip. (*A*)	^{3}H-halo. (*B*)	^{3}H-spip. (*C*)	^{3}H-LSD (*D*)	Apomorphine stereotypy (*F*)	Tryptamine seizures (*F*)	*In vitro*† activities	*In vivo* activities *E*/*F*
Pipamperone	5.9±0.1	6.5±0.2	7.79±0.06	7.57±0.07	14.3	1.55	19.5	9.2
Clozapine	5.9±0.2	6.4±0.2	7.32±0.09	7.08±0.07	32.7	9.39	8.3	3.5
Azaperone	6.50±0.05	6.7±0.2	7.45±0.06	7.5±0.1	2.35	1.35	5.6	1.7
Chlorprothixene	7.25±0.05	7.55±0.05	8.00±0.05	7.61±0.09	1.08	1.45	2.8	0.7
Clothiapine	7.1±0.1	7.4±0.2	7.74±0.08	7.4±0.1	0.38	1.11	2.2	0.3
Chlorpromazine	6.85±0.07	6.9±0.1	7.21±0.07	6.6±0.1	0.82	2.48	2.0	0.3
Methithepine	8.0±0.2	8.0±0.1	8.23±0.07	7.95±0.05	0.20	0.20	1.7	1.0
Propericiazine	7.90±0.05	8.0±0.1	8.1±0.1	7.2±0.1	0.23	0.90	1.3	0.3
Flupenthixol	7.23±0.05	7.7±0.2	7.40±0.05	6.75±0.05	0.26	0.82	0.5	0.3
Thioridazine	6.65±0.05	7.4±0.2	6.96±0.07	6.8±0.1	13.1	26.3	0.4	0.5
Fluspiperone	8.74±0.04	9.4±0.1	8.68±0.05	8.1±0.1	0.032	0.12	0.2	0.3
Fluphenazine	7.41±0.07	7.8±0.2	7.0±0.1	6.6±0.1	0.13	1.14	0.2	0.1
Droperidol	8.1±0.1	8.7±0.1	7.90±0.05	7.57±0.07	0.035	1.29	0.2	0.03
Spiperone	8.90±0.03	9.4±0.1	8.45±0.05	8.2±0.1	0.053	0.21	0.1	0.2
(+)-Butaclamol	7.7±0.1	8.0±0.2	7.0±0.1	7.38±0.06	0.28	1.68	0.1	0.2
Trifluperidol	7.85±0.06	8.8±0.1	7.55±0.05	6.90±0.05	0.040	0.74	0.06	0.05
Benperidol	8.35±0.06	9.05±0.05	7.70±0.05	7.6±0.1	0.023	0.76	0.04	0.03
Pimozide	7.80±0.05	8.5±0.1	7.0±0.1	6.7±0.1	0.10	>22	0.03	0.004
Clopimozide	7.03±0.06	8.1±0.3	6.5±0.1	6.45±0.05	0.30	>80	0.03	0.004
Haloperidol	7.69±0.06	8.5±0.1	6.83±0.07	6.15±0.05	0.043	2.05	0.02	0.02
Oxiperomide	7.7±0.4	7.93±0.08	6.05±0.05	6.35±0.05	0.11	7.95	0.01	0.01
Metoclopramide	5.8±0.1	6.95±0.05	4.93±0.08	5.15±0.05	1.69	41.4	0.01	0.04
Sulpiride	6.06±0.06	7.1±0.1	4.1±0.1	<5	62.8	938	0.001	0.07

In this series of compounds statistically significant correlations ($P < 0.01$) were obtained between the activities of *A* and *B* ($r = 0.92$), *C* and *D* ($r = 0.93$), *A* and *E* ($r = 0.81$), *A* and *E*+*F* ($r = 0.84$), *B* and *E* ($r = 0.85$), *C* and *F* ($r = 0.83$), *D* and *F* ($r = 0.74$).

*Mean values were calculated from three independent determinations using freshly prepared tissue and drug solutions, and performed in duplicate.

Presently shown IC_{50} values were not converted into K_i values using the Cheng-Prusov equation, since both receptor populations in striatum and frontal cortex seemed to be heterogeneous and the real dissociation constant of the labelled ligand cannot be measured.

†Ratio of *in vitro* activities is calculated as IC_{50} (halo., striatum)/IC_{50} (spip., frontal cortex), expressing within the series for the various compounds differences in dissociation between affinity for frontal cortical and striatal receptors.

pimozide and clopimozide[14,15], were 30 to 40 times more active inhibitors of haloperidol binding in the striatum than of spiperone binding in the frontal cortex.

Regression analysis (correlation coefficients in Table 2) established that *in vitro* binding potency in the frontal cortex was highly correlated with *in vivo* potency in the antagonism of bilateral clonic seizures induced by tryptamine. The IC_{50} values of the *in vitro* striatal binding tests were correlated with *in vivo* antagonism of apomorphine stereotypy. Also noteworthy is the highly significant correlation shown by means of the two-variable regression equation which relates spiperone binding in the striatum to anti-apomorphine and anti-tryptamine activity simultaneously [$-\log IC_{50}(A) = -0.54 \log ED_{50}(E) - 0.33 \log FD_{50}(F) + 7.2$] ($r = 0.84$, $P < 0.01$) (see Table 2 for significance of *A*, *E*, *F*). A similar multivariable correlation could not be obtained between any of the other binding data.

The *in vitro* finding that neuroleptics bind to both striatal and frontal cortex receptors, and that certain compounds are more selective for one brain area was further confirmed by *in vivo* binding studies (Table 3). *In vivo* displacement of ^{3}H-spiperone from its receptors by pipamperone and LSD was readily obtained at low doses in the frontal cortex, but not in the brain

Table 3 *In vivo* displacement of labelled spiperone in rat brain

Region	Control	Labelled spiperone (pg mg^{-1})				
		LSD		Pipamperone		Haloperidol
		0.63 mg kg^{-1}	10 mg kg^{-1}	0.63 mg kg^{-1}	10 mg kg^{-1}	2.5 mg kg^{-1}
After 2 h						
Striatum	3.8±0.1	3.7±0.1	3.2±0.1†	4.0±0.3	3.6±0.1	2.9±0.2‡
Nucleus accumbens	3.6±0.2	3.5±0.3	3.2±0.1	3.5±0.3	3.2±0.2	2.7±0.0*
Tuberculum olfactorium	2.5±0.1	2.5±0.2	2.0±0.2*	2.1±0.3	2.2±0.2	2.2±0.1*
Frontal cortex	1.5±0.04	0.8±0.1‡	0.65±0.02‡	0.94±0.02‡	0.65±0.02‡	0.83±0.02‡
Cerebellum	0.40±0.03	0.37±0.01	0.40±0.01	0.38±0.02	0.36±0.02	0.35±0.01
After 4 h						
Striatum	3.7±0.1	4.3±0.3	3.8±0.1	3.8±0.1	2.7±0.1‡	2.1±0.1‡
Nucleus accumbens	3.4±0.2	3.9±0.3	3.0±0.1	3.2±0.1	2.4±0.3*	2.1±0.1†
Tuberculum olfactorium	2.2±0.1	2.1±0.2	1.9±0.1	2.2±0.2	1.5±0.1‡	1.4±0.1‡
Frontal cortex	0.8±0.1	0.48±0.02‡	0.43±0.03‡	0.62±0.06*	0.37±0.01‡	0.44±0.01‡
Cerebellum	0.27±0.02	0.22±0.01	0.25±0.01	0.30±0.03	0.23±0.01	0.27±0.01

Rats were given ^{3}H-spiperone (0.005 mg kg^{-1}, specific activity 9 Ci mmol^{-1}) intravenously. One hour later, unlabelled LSD, pipamperone and haloperidol were similarly injected. Two and four hours after the labelled drug, radioactivity was determined in various brain regions. Each value is the mean of four determinations ± s.e.m. Significant differences (by Student's *t* test) from control values are indicated *$P < 0.05$ †$P < 0.01$ ‡$P < 0.001$.

dopaminergic areas. Radioactivity in dopaminergic areas was reduced by high doses of LSD, but only significantly after 2 h. In contrast, haloperidol supplanted ^{3}H-spiperone in both dopaminergic areas and in the frontal cortex.

These results show that spiperone and LSD label the same receptors in the frontal cortex and that these receptors are different from those labelled by spiperone and haloperidol in the striatum. In *in vitro* as well as *in vivo* binding experiments, neuroleptics showed high affinity for the receptors in both brain areas. Different observations provided evidence that receptors in the frontal cortex are predominantly of serotonergic nature: the receptors could be differentiated by serotonin agonists and antagonists; LSD has previously been described as a ligand for serotonergic receptors[16]; and a direct correlation has been shown between relative affinities for frontal cortical receptors and *in vivo* antagonism of the tryptamine test. Tryptamine is assumed to mimic serotonin action at central and peripheral receptor sites[17,18].

In confirmation of previous reports, striatal receptors seemed to be chiefly dopaminergic, as evidenced by the correlation between striatal *in vitro* binding activity and the *in vivo* potency in the apomorphine test. It has frequently been reported that apomorphine mimics central dopaminergic effects (see refs in ref. 19). At present there is little evidence of the homogeneity or heterogeneity of the receptor populations in the brain areas studied. Kinetic investigations have indicated the presence of different binding sites in the striatum[4], and these were more apparent with ^{3}H-spiperone than with ^{3}H-haloperidol. The two-variable regression equation presented here (between A and $E+F$) also points to a minor serotonergic component in the striatal binding sites. This is supported by the findings of Withaker and Seeman[20] that hallucinogens bind to neuroleptic receptors in the calf caudate. The serotonergic component, however, did not become apparent in the binding of ^{3}H-haloperidol in the striatum, therefore this test was chosen more as a pure *in vitro* assessment of the anti-dopaminergic activity of drugs. Investigations of the kinetics of ^{3}H-spiperone binding in the frontal cortex also pointed to the presence of various different binding sites (our work, in preparation).

The experiments reported here are the first *in vitro* demonstration that both serotonergic and dopaminergic receptors may be involved in the mechanism of action of neuroleptics. The degree of affinity and the ratio of affinity of the drugs for receptors is closely reflected in their pharmacological potency and profile. The precise significance of the anti-serotonergic component of neuroleptics, however, requires assessment of its clinical implications. Note that pipamperone, the compound with the highest dissociation in binding affinity in favour of the frontal cortex, is noted for its particular properties in clinical psychiatry. The drug is known for its anti-agitation effects[21–24] and its conspicuous capacity to normalise disturbed sleep rhythms in psychiatric patients[25]. Pimozide, however, which had very pronounced striatal activity, is a potent anti-psychotic with a profile which is different from other neuroleptics[26]. The present observations do not detract from the dopamine theory on schizophrenia, but they do imply that a broadening of this theory to cover serotonergic processes may increase our understanding of this complexity of disorders.

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Functions of calcium in sweat secretion

CALCIUM is an important regulatory factor in many physiological systems, including fluid-transporting epithelia. There is good evidence that such regulatory factors are involved in normal secretory function[1], and indirect evidence that they may be involved in secretory irregularities in disease states[2,3]. Using a novel *in vitro* preparation, we have examined the effects of calcium on the secretory properties of the human eccrine sweat gland. The sweat gland consists of a single coiled tubule which is closed at the proximal secretory end and open at the distal re-absorptive end. On stimulation, the secretory portion of the tubule secretes an isotonic precursor fluid from which NaCl is absorbed hypertonically in the distal, re-absorptive duct. The final secretory product, hypotonic sweat, is excreted on to the epidermis for evaporation. We have found that calcium is an essential requirement for stimulating sweat and that this ion may be a factor in regulating the hypotonicity of sweat.

Skin biopsies were taken from the upper back of 21–39-yr-old male volunteers with an electric biopsy punch 3 mm in diameter. The skin plugs were mounted in 15-ml chambers such that the epidermis was continuously immersed in hexadecane while the dermal and subdermal tissues were bathed in oxygenated Ringer's solution (132 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 11 mM glucose, 5 mM NaHEPES and $CaCl_2$ to the desired concentration; pH 7.4). With this arrangement, small droplets of sweat formed under the oil on the epidermis when the sweat glands were stimulated pharmacologically by adding 5×10^{-7} M mecholyl to the bath. The droplets from active glands (one to three per biopsy plug) were collected in constant bore capillaries between oil blocks. Secretory rates of single glands were measured by dividing the sample volume by the time in minutes during which the sample was secreted (usually 2–5 min). The concentrations of sodium and calcium were determined by microdroplet X-ray analysis[4]. The preparation will be described in detail elsewhere.

As shown in Table 1, secretory rates at a bath calcium concentration of 5.0 and 10.0 mM were significantly lower ($P < 0.05$) than at 2.0 mM, suggesting that at higher extracellular concentrations, calcium may be somewhat inhibitory to active secretion or that it may be acting to decrease the epithelial permeability such that the passive movements of fluid and electrolytes are restricted[5].

However, secretory activity was found to be entirely dependent on the presence of external calcium in the bath (Table 1). Sweat