POSTER COMMUNICATIONS

Interaction of N-acyl and N-sulphonyl derivatives of homotaurine (3-aminopropanesulphonic acid) with GABA receptor sites and their effects on the central nervous system of the rat

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Homotaurine is a GABA receptor agonist (Enna & Snyder, 1975) endowed with anticonvulsant activity in different experimental models (Adembri, Bartolini, Bartolini, Giotti & Zilletti, 1974). In an attempt to develop new GABAergic drugs, we evaluated the relative potencies of some N-substituted homotaurine derivatives as inhibitors of [^{3}H]-GABA receptor binding in synaptic membranes of rat brain. The same compounds were tested for neurological activity after intraventricular and intranigral injection in the rat.

[^{3}H]-GABA binding was measured by the basic method of Enna & Snyder (1975). In our experiments, however, membranes were pretreated with Triton X-100 (0.01%) and washed three times with buffer prior to being frozen and stored for binding assays.

Turning behaviour was tested according to Arnt, Scheel-Krüger, Magelund & Krogsgaard-Larsen (1979).

The results obtained (Table 1) show that the introduction of acyl or sulphonyl groups on the N atom of homotaurine has variable effects on drug–receptor interaction: while compounds III, V and VI maintain a remarkable affinity for GABA receptor sites, compound IV is practically devoid of such action.

As for the neurological effects, when these compounds were injected intraventricularly in the rat, compounds III and IV were ineffective, while V and, to a lesser extent, VI, induced rather severe convulsive syndromes. In the latter compounds, therefore, the N-sulphonyl substituents gave rise to neurological activity opposite to that of homotaurine. After intranigral injection, compounds III, IV and VI showed no effect, while homotaurylhomotaurine (V) induced strong ipsilateral turning behaviour.

This preliminary study shows that some N-sulphonyl derivatives of homotaurine maintain an affinity for GABA receptor sites, although in some cases acquiring antagonistic action.

Table 1

		[^{3}H]-GABA binding IC_{50} (μM)	Convulsive syndrome	Turning behaviour
I	GABA $H_2N(CH_2)_3COOH$	0.015 ± 0.006	0	Contralateral
II	Homotaurine H—R	0.020 ± 0.008	0	Contralateral
III	Acetylhomotaurine CH_3CO—R	1.3 ± 0.09	0	0
IV	Carbobenzoxyhomotaurine $C_6H_5CH_2OCO$—R	1000 ± 212	0	0
V	Homotaurylhomotaurine $H_2N(CH_2)_3SO_2$—R	0.30 ± 0.06	+ + +	Ipsilateral
VI	Carbobenzoxyhomotaurylhomotaurine $C_6H_5CH_2OCONH(CH_2)_3SO_2$—R	0.19 ± 0.04	+ +	0
VII	Muscimol	0.004 ± 0.001	0	Contralateral
VIII	Bicuculline M C	7.0 ± 2.0	+ + +	Ipsilateral

R = —$NH(CH_2)_3SO_3H$.
IC_{50} values were derived graphically from log-probit plots of 3 to 5 different concentrations of the compounds, each assayed in triplicate; data represent mean ± s.e. mean of 3–4 independent determinations.

References

ADEMBRI, G., BARTOLINI, A., BARTOLINI, R., GIOTTI, A. & ZILLETTI, L. (1974). Anticonvulsive action of homotaurine and taurine. *Br. J. Pharmac.*, **52**, 439–440P.

ARNT, J., SCHEEL-KRÜGER, J., MAGELUND, G. & KROGSGAARD-LARSEN, P. (1979). Muscimol and related GABA receptor agonists: the potency of GABAergic drugs *in vivo* determined after intranigral injection. *J. Pharm. Pharmacol.*, **31**, 306–313.

ENNA, S.J. & SNYDER, S.H. (1975). Properties of γ-aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fraction. *Brain Res.*, **100**, 81–97.

A radioreceptor assay using [^{3}H]-muscimol for GABA in human cerebrospinal fluid

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There is evidence that abnormalities in GABA neurotransmission may underlie certain neurological disorders. Thus, for example, GABA concentrations in CSF of patients suffering from Huntington's Chorea have been reported to be significantly lower than in control subjects (Enna, Stern, Wastek & Yamamura, 1977), and animal studies suggest that CSF levels of this amino acid closely reflect central GABA fluctuations (Bohlen, Huot & Palfreyman, 1979).

Most previous assays for GABA in CSF have utilised radioreceptor techniques with [^{3}H]-GABA as radioligand (Enna, Wood & Snyder, 1977). However, since muscimol, a specific GABA agonist, has been shown to possess an even higher affinity for GABA receptors than GABA itself (Williams & Risley, 1979) we have developed a sensitive assay using [^{3}H]-muscimol and have estimated GABA in CSF of 12 patients.

Whole pig brain was homogenised in 50 mM Tris-HCl buffer, pH 7.8, and washed in 0.05% Triton X-100. The membranes were subsequently washed a further seven times in Tris buffer to remove the detergent and remaining endogenous GABA, and then frozen at −60°C. Immediately prior to the assay the membranes were thawed and washed again in buffer. Specific [^{3}H]-muscimol binding (binding displaced by 200 μM GABA) was saturable and Scatchard and Hill analyses indicated interaction with a single population of sites in these membranes (K_d 24.5 nM; B_{max} 2.82 pmol/mg protein). [^{3}H]-GABA binding to the same membranes was considerably less (K_d 52.6 nM; B_{max} 1.66 pmol/mg protein). At the K_d, the ratio of specific to non-specific binding was also higher (9:1) with [^{3}H]-muscimol than with [^{3}H]-GABA (7:3) emphasising the more favourable properties of [^{3}H]-muscimol in a radioreceptor assay.

In the assay all constituents were diluted in 50 mM Tris-HCl, pH 7.8, and membranes (0.5 to 0.7 mg protein) were incubated for 30 min at room temperature with 5 mM [^{3}H]-muscimol in a total volume of 250 μl. Bound [^{3}H]-muscimol was separated from free ligand by filtration through Whatman GF/B filters. Log-probit analysis demonstrated that GABA displaced [^{3}H]-muscimol with high affinity (IC_{50} 20 pmol) and the sensitivity of the assay (20% inhibition of [^{3}H]-muscimol binding) was 5 pmol.

CSF was obtained by lumbar puncture from fasting patients, recumbent for 12 h prior to the procedure. All were free of organic neurological disease. The specimens were rapidly frozen and stored at −60°C. Assays were performed in triplicate on untreated CSF samples and mean GABA concentration for 12 patients was 278 ± 33 pmol/ml.

We are at present using this assay to investigate patients suffering from various neurological disorders.

References

BOHLEN, P., HUOT, S. & PALFREYMAN, M. (1979). The relationship between GABA concentrations in brain and CSF. *Brain Research*, **167**, 297–305.

ENNA, S.J., STERN, L.Z., WASTEK, G.J. & YAMAMURA, H.I. (1977). Cerebrospinal fluid γ-aminobutyric acid variation in neurological disorders. *Arch. Neurol.*, **34**, 683–685.

ENNA, S.J., WOOD, J.H. & SNYDER, S.H. (1977). γ-Aminobutyric and GABA in human CSF radioreceptor assay. *J. Neurochem.*, **28**, 1121–1124.

WILLIAMS, M. & RISLEY, E.A. (1979). Characterisation of the binding of [^{3}H]-muscimol, a potent GABA agonist, to rat brain synaptosomal membranes using a filtration assay. *J. Neurochem.*, **32**, 713–718.

Serotonin recognition sites are labelled in cerebral cortex by the β-adrenoceptor antagonist [^{125}I]-hydroxybenzylpindolol

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[^{125}I]-hydroxybenzylpindolol ([^{125}I]-HYP) has been widely used to identify β-adrenoceptors in many tissues and because of its much higher specific activity possesses a clear advantage over [^{3}H]-β-adrenoceptor ligands (Maguire, Ross & Gilman, 1977). In this communication, however, we report that [^{125}I]-HYP may label serotonin recognition sites in brain and that this property severely hampers an accurate analysis of cerebral β-adrenoceptors with this ligand.

Rat erythrocyte membranes were prepared essentially as described by Charness, Bylund, Beckman, Hollenberg & Snyder (1977) and rat cerebral cortical membranes as previously described (Nahorski, 1978). [^{125}I]-HYP binding was assayed using established techniques (Nahorski, 1978) except that membranes were washed during filtration with 25 mM Tris-HCl, 140 mM NaCl, pH 7.8.

Specific [^{125}I]-HYP binding to rat erythrocyte membranes (binding displaced by (−)-isoprenaline (200 μM) was saturable (B_{max} 132 ± 4.6 fmol/mg protein), of high affinity (K_d 58 ± 4.5 pM) and displaced by a variety of β-adrenoceptor agonists and antagonists with affinities indicative of a sole interaction with a classical β-adrenoceptor. The pharmacological profile of [^{125}I]-HYP binding to rat erythrocyte membranes is identical to that seen with another ligand, [^{3}H]-dihydroalprenolol (Dickinson & Nahorski, 1979). In contrast, [^{125}I]-HYP binding to rat cerebral cortical membranes differed substantially to that seen in erythrocytes in that β-adrenoceptor agents such as (−)-isoprenaline, (−)-propranolol, (±)-atenolol and (±)-HYP displaced the binding of 60 to 80 pM [^{125}I]-HYP in a biphasic manner. Only 30 to 40% of the total binding possessed properties indicative of a true β-adrenoceptor. The remaining sites were examined by performing incubations in the presence of (−)-isoprenaline (30 μM) to eliminate binding to β-adrenoceptors. 5-Hydroxytryptamine (5-HT) and related compounds displaced [^{125}I]-HYP binding from these sites (IC_{50}—5-HT, 4 μM; tryptamine, 10 μM; 5-methoxytryptamine, 200 μM; 5,6-dihydroxytryptamine, 1000 μM; 5-hydroxyindoleacetic acid > 1000 μM; indole > 1000 μM). Also several β-adrenoceptor antagonists displaced this binding, though with lower affinity and less stereoselectivity than that expected at a β-adrenoceptor (IC_{50}—(±)-HYP, 30 nM; (−)-propranolol, 290 nM; (+)-propranolol, 2000 nM). However, (±)-atenolol was virtually inactive (IC_{50} > 1000 μM).

Our findings suggest that whereas [^{125}I]-HYP binds solely to β-adrenoceptors on erythrocyte membranes, it labels both β-adrenoceptors and sites that recognise 5-HT-like compounds in cerebral cortex. It is of interest that some β-adrenoceptor antagonists also inhibit binding to these latter sites and it would seem that our findings may relate to inhibitory effects of some β-blockers on [^{3}H]-5-HT binding in cerebral tissue (Middlemiss, Blakeborough & Leather, 1977). Although at the present time it would be premature to suggest that [^{125}I]-HYP labels 5-HT receptors in brain, the results are of interest since [^{125}I]-HYP possesses an indole moiety and emphasises the difficulties inherent in using this ligand to label β-adrenoceptors in brain.

References

CHARNESS, M.E., BYLUND, D.B., BECKMAN, B.S., HOLLENBERG, M.D. & SNYDER, S.H. (1976). Independent variation of β-adrenergic receptor binding and catecholamine stimulated adenylate cyclase activity in rat erythrocytes. *Life Sci.*, **19**, 243–250.

DICKINSON, K.E.J. & NAHORSKI, S.R. (1979). Homogeneity of β-adrenoceptors on rat erythrocytes. *Brit. J. Pharmac.*, **67**, 479–480P.

MAGUIRE, M.E., ROSS, E.M. & GILMAN, A.G. (1977). β-Adrenergic receptors: ligand binding properties and the interaction with adenylyl cyclase. *Adv. Cycl. Nucl. Res.*, **8**, 1–83.

MIDDLEMISS, D.N., BLAKEBOROUGH, L. & LEATHER, R.S. (1977). Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor. *Nature, Lond.*, **267**, 289–290.

NAHORSKI, S.R. (1978). Heterogeneity of cerebral β-adrenoceptor binding sites in various vertebrate species. *Eur. J. Pharmacol.*, **51**, 199–209.

Differences in the development of tolerance to morphine and D-ala^2-methionine-enkaphalin in two inbred strains of mice

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Stereospecific opiate binding sites have been discovered and mapped in the brain and endogenous peptides with strong opiate agonist properties have been isolated and identified (Pert & Snyder, 1973; Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975). As an important step in the elucidation of the mechanism of action of these peptides, the development of tolerance and cross tolerance between morphine and methionine-enkephalin (met-enk) has been described (Wei & Loh, 1976).

We have recently demonstrated that met-enk resembles morphine in the strain specificity of the analgesic response in C57 BL/6J (C57) and DBA/2J (DBA) mice (Racagni, Frigeni, Bruno, Carenzi & Santini, 1978). In fact, met-enk, like morphine, was more potent in eliciting analgesia in DBA than in C57 strain, in which only morphine exerted a clear stimulating effect on locomotor activity (Oliverio & Castellano, 1974; Racagni, Bruno, Iuliano & Paoletti, 1979). Moreover, we have shown that different neurochemical mechanisms underly the difference in the sensitivity of C57 and DBA mice to the effect of a single injection of morphine (Racagni *et al.* 1979). It was, therefore, of interest to compare the effects of met-enk with those of morphine during the onset and offset of tolerance to analgesia in C57 and DBA mice.

Tolerance was produced by injecting mice systemically (s.c.) with the corresponding ED_{50} of morphine for analgesia. The degree of analgesia was assessed with either morphine or D-ala^2-met-enk injected intracerebroventricularly (i.c.v.). The challenging dose of morphine i.c.v. (0.25 μg for DBA and 1.0 μg for C57 mice) and the challenging dose of D-ala^2-met-enk i.c.v. (0.03 μg for DBA and 0.12 μg for C57 mice), corresponding to the ED_{50} for analgesia, were administered after 1, 4 and 8 morphine s.c. injections.

The degree of tolerance was determined by the hot plate method. The results show similar differences in analgesic activity following i.c.v. injections of D-ala^2-met-enk in the two strains of mice chronically treated with morphine s.c. On the other hand, a different pattern of response was found when the challenging ED_{50} for analgesia of morphine was administered i.c.v. to the two strains of mice chronically pretreated with morphine s.c. Indeed, C57 strain, unlike DBA, developed tolerance immediately after the first s.c. morphine pretreatment, whereas in DBA mice the rate of decrease in potency appeared upon eight morphine administrations.

These results demonstrate that in the C57 strain the onset of the tolerance is much faster than in the DBA mice. Studies on the offset of the tolerance demonstrate that the C57 strain is resistant to the analgesic effect of morphine and D-ala^2-met-enk.

The different sensitivity of the two strains of mice in the development of tolerance after the administration of the opiate alkaloid and the endogenous opiate suggest that genetic mechanisms play an important role in modulating the effects of morphine and enkephalins on behaviour, thus confirming the significance of using inbred strains in the evaluation of the mode of action of analgesic compounds.

References

HUGHES, J., SMITH, T.W., KOSTERLITZ, H.W., FOTHERGILL, L.A., MORGAN, B.A. & MORRIS, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature*, **258**, 577–579.

OLIVERIO, A. & CASTELLANO, C. (1974). Genotype dependent sensitivity to morphine and heroin. *Psychopharmacology*, **39**, 13–22.

PERT, C.B. & SNYDER, S.H. (1973). Properties of opiate-receptor binding in rat brain. *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2243–2247.

RACAGNI, G., BRUNO, F., IULIANO, E. & PAOLETTI, R. (1979). Differential sensitivity to morphine-induced analgesia and motor activity in two inbred strains of mice: behavioural and biochemical correlations. *J. Pharmacol. Exp. Ther.*, **209**, 111–116.

RACAGNI, G. FRIGENI, V., BRUNO, F., CARENZI, A. & SANTINI, V. (1978). Analgesia and motor activity elicited by morphine and enkephalins in two inbred strains of mice. *J. Pharm. Pharmacol.*, **30**, 310–14.

WEI, E. & LOH, H. (1976). Physical dependence on opiate-like peptides. *Science*, **193**, 1262–63.

Effect of hyperprolactinemia on β-endorphin and met-enkephalin concentrations in brain and pituitary

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The effects of several hyperprolactinaemic conditions on β-endorphin and met-enkephalin concentrations in brain regions and the pituitary have been investigated in famale rats.

In Buffalo rats bearing a prolactin and adrenocorticotrophin secreting tumour (7315a) for 5 weeks, a decrease ($P < 0.01$) in β-endorphin, but not in met-enkephalin, concentrations was present in the hypothalmus, midbrain, hindbrain and pituitary when compared to intact rats. In rats bearing the tumour for 3 weeks, a similar pattern was present with the exception of the midbrain, in which no change was evident. In Wistar Furth rats bearing a prolactin and growth hormone secreting tumour (MtTW15) for 8 weeks, a decrease ($P < 0.01$) in β-endorphin concentrations in the hypothalamus and midbrain was present. Met-enkephalin concentrations decreased ($P < 0.02$) in the hypothalamus, midbrain and hindbrain. In rats bearing the tumour for 5 weeks, the decrease ($P < 0.01$) in β-endorphin concentrations was similar in magnitude.

In rats bearing two pituitaries transplanted under the kidney capsule for 5 days, no change in β-endorphin or met-enkephalin concentrations was observed. When four pituitaries were transplanted and the experiments conducted four weeks later, a decrease ($P < 0.05$) in β-endorphin concentrations was present in the hypothalamus and hindbrain.

In lactating rats eight day postpartum, a decrease ($P < 0.01$) in β-endorphin concentrations was present in the hypothalamus and midbrain. Met-enkephalin decreased ($P < 0.01$) in the hypothalamus, midbrain and hindbrain. β-Endorphin concentrations were not changed in rats weaned 8 days or 2 days after 6 days of lactation. A decrease ($P < 0.05$) in β-endorphin concentrations in the pituitary was present immediately after delivery, while no change was observed in the brain. Concentrations of endogenous opiates did not change in the brain regions of rats bearing tumours secreting other pituitary hormones.

In conclusion: (1) prolactin influences β-endorphin and met-enkephalin concentrations in the brain; (2) the duration of hyperprolactinemia is critical for the effects on β-endorphin and met-enkephalin brain concentrations; (3) β-endorphin and met-enkephalin concentrations do not always show similar patterns.

Behavioral effects of chlopromazine and chlordiazepoxide in rats: qualitative or quantitative differences?

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Neuroleptics seem to influence mainly those behaviours which are maintained by presentation of positive reinforcers or withdrawal of negative reinforcers, whereas anxiolytics appear to affect especially those behaviours maintained by presentation of negative reinforcers or withdrawal of positive reinforcers (Stein, Belluzzi & Wise, 1977). However, several contradictory data have appeared in the literature and, moreover, conclusions have been drawn from experiments different one from another in several respects (kind of positive reinforcers, features of shock, animal species, and so on).

In order to overcome these difficulties in the present study drug effects have been analyzed in the same animal (rat) using a multiple-concurrent schedule of reinforcement encompassing the four events resulting from presentation or withdrawal of positive or negative reinforcers. The schedule was as follows: when a pilot light was off, a 2' VI schedule was in operation at the left lever and a CRF-punished schedule was concurrently in operation on the right lever; when the pilot light was on, the program was switched to an extinction schedule on the left lever and to a Sidman avoidance schedule on the right lever. Under control conditions animals worked mainly on the left lever during dark periods and on the right lever during light periods.

Groups of 5 to 11 animals were treated i.p. with chlorpromazine (0.5, 1, 2 and 4 mg/kg) or with chlordiazepoxide (1.25, 2.5, 5, 10 and 20 mg/kg). It was found that chlordiazepoxide increased in punished behaviour in a dose related manner (minimal effective

dose 2.5 mg/kg), whereas the effects of chlorpromazine were inconsistent since only one dose (2 mg/kg) was effective. On the other hand the two drugs had qualitatively similar effects upon approach, extinction and avoidance behaviour, but chlorpromazine influenced these behaviours approximately at the same dose, while chlordiazepoxide was more potent upon extinction than upon approach or avoidance.

The results point out that a multiple-concurrent schedule can give a better characterization of the effects of psychotropic drugs upon behaviour.

Reference

STEIN, L., BELLUZZI, J.D. & WISE, C.D. (1977). The physiology of operant behavior. In *Animal Models in Psychiatry and Neurology*, eds. Hanin and Usdin, pp. 9–16. Oxford: Pergamon Press.

Behavioral effects induced by low doses of methylmercury in rats

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Methylmercury may induce subtle behavioural effects at dose levels below those associated with overt symptoms of neurotoxicity (Spyker, 1975).

Since it would seem that behavioural techniques offer possibilities for the assessment of effects that are not available by morphological and biochemical approaches (Kishi, Hashimoto, Shimzu & Kobayashi, 1978), the purpose of the present investigation was to investigate the effects of low doses of methylmercury chloride (MMC) on locomotor activity and avoidance performance in rats.

The experiments were carried out on 90 naive male Sprague–Dawley rats weighing 250 to 280 g. Locomotion was evaluated by using a series of toggle-floor boxes; for each rat the number of crossings performed during a 10-min session was recorded. A single injection of MMC (0.25 or 0.5 mg/kg/i.p.) dissolved in saline was given 1 h, 10, 20 and 30 days before the test session, respectively.

Avoidance performance was evaluated by using a series of shuttle-boxes. Rats were subjected to daily 20-trial training sessions. When the performance of animals was definitively stable, they were treated with a single dose of MMC (0.25 or 0.5 mg/kg/i.p.) 1 h before the test session. Thereafter rats were subjected to 30 daily conditioning sessions.

Our results show a significant depression of locomotor activity only during the session performed 1 h after the administration of 0.25 ($P < 0.05$) and 0.5 ($P < 0.01$) mg/kg of MMC.

For avoidance performance, there was a decrement of avoidance responses ($P < 0.05$) during the session performed 1 h after the injection of MMC (0.5 mg/kg).

This initial impairment of avoidance responses completely disappeared in the next six conditioning sessions.

Beginning at the seventh session, there were notable changes in day to day avoidance behavior of MMC (0.5 mg/kg)-treated group. Particularly, animals exhibited an increase in the variability of avoidance performance which lasted until the nineteenth session. These delayed effects induced by MMC were not found in the locomotor activity level nor in motor co-ordination. Further, there were no significant differences between treated and non-treated animals in terms of body weight.

In conclusion, our results provide further evidence that doses of MMC, which are too low to yield gross CNS disturbances, can induce delayed behavioural changes which can be accurately displayed by using active avoidance procedures.

References

KISHI, R., HASHIMOTO, K., SHIMIZU, S. & KOBAYASHI M. (1978). Behavioural changes and mercury concentrations in tissues of rats exposed to mercury vapour. *Toxicol. and Appl. Pharmacol.*, **46**, 555–566.

SPYKER, J.M. (1975). Behavioural teratology and toxicology. In *Behavioural Toxicology*. eds. Weiss, B. & Laties, V.G. pp. 311–344. New York: Plenum Press.

Neurotoxic lesion of the median raphe nucleus protects rats from electroconvulsive shock-induced retrograde amnesia of passive avoidance conditioning

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Retrograde amnesia (RA) induced by electroconvulsive shock (ECS) is thought to be due to a sudden release of brain 5-hydroxytryptamine (5-HT) followed by reduced brain protein synthesis (Essman, 1973). Indeed we have observed an early and short-lasting increase in brain 5-HT turnover following a single ECS (Gandolfi, Dall'Olio & Montanaro, 1978), as well as protection from ECS-induced RA of passive avoidance (PA) conditioning by i.p. pretreatment of rats with the 5-HT receptor antagonists bromolysergide and methysergide (Montanaro, Dall'Olio & Gandolfi, 1979). Memory consolidation therefore appears to be inversely related to serotonergic neurotransmission.

In order to validate such interpretation, the amnestic effect of ECS has been assayed in rats with neurotoxic lesion of the median raphe nucleus (MRN), whose projections are mainly towards the hippocampus, a structure playing a prominent role in learning and memory. MRN of 40 male Sprague–Dawley rats was stereotactically injected with 5,7-dihydroxytryptamine (28 μg/2 μl/rat, as free base) 50 min after desimipramine (25 mg/kg, i.p.); 40 rats received solvent (sham lesioned). Fifteen days after surgery, the rats were submitted to one trial step-down PA conditioning by using the procedure and apparatus previously described (Montanaro *et al.*, 1979). Five sec after footshock, half of the animals of each group received ECS (400 ms, 1000 V, 50 mA) through ear clips. The remaining rats were submitted to sham-ECS. Memory test (24 h later) consisted of measurement of the time spent by the animals on the platform.

Results from ANOVA performed on reciprocal-transformed training and test latencies were interpreted (Li, 1964) in terms of comparisons among the median values (given in brackets below) in the original scale. Training session stepping-down was significantly ($P < 0.002$) shorter in MRN-lesioned rats (4.5 s) in comparison to controls (8.5 s). Among sham-lesioned rats, controls exhibited a significant ($P < 0.001$) PA conditioning (training: 8.3 s; test: 53.5 s), whereas those submitted to ECS showed a clear-cut RA (training: 8.5 s; test: 15.3 s; N.S.). On the contrary, both sham-ECS (>120 s) and ECS (60.0 s) MRN-lesioned groups showed significantly ($P < 0.001$) longer durations on the platform on test session in comparison to their training values (4.7 s and 3.2 s, respectively), thus indicating that ECS resulted ineffective as amnestic agent after MRN lesion.

The shorter spontaneous stepping-down observed in MRN-lesioned rats might be interpreted by considering that unpunished response is sustained by two opposite drives (curiosity and fear of descending): lesion of 5-HT ascending pathways might have diminished the inhibitory component of such spontaneous behaviour.

On the other hand, MRN lesion could have produced a lower synaptic availability of 5-HT at the time of ECS administration, thus preventing ECS from initiating the neurochemical processes related to memory consolidation impairment.

References

ESSMAN, A.B. (1973). Experimentally induced retrograde amnesia: some neurochemical correlates. In *Current biochemical approaches to learning and memory*. eds. Essman and Nakajima. pp. 159–188. New York: Spectrum Publications, Inc.

GANDOLFI, O., DALL'OLIO, R. & MONTANARO, N. (1978). Early changes in rat brain 5-hydroxytryptamine synthesis after a single electroconvulsive shock. *Pharmac. Res. Commun.*, **10**, 75–79.

LI, J.C.R. (1964). Statistical Inference. Edwards Brothers, Inc., Ann Arbor.

MONTANARO, N., DALL'OLIO, R. & GANDOLFI, O. (1979). Bromolysergide and methysergide protection against ECS-induced retrograde amnesia. *Neuropsychobiology*, **5**, 174–180.

Role of brain acetylcholine in reflex control in the conscious rat

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Experiments were performed in unanesthetized rats to determine whether CNS cholinergic mechanisms are involved in reflex-induced bradycardia and tachycardia. Baroreceptors were activated with i.v. bolus injections of noradrenaline (NE) or sodium nitroprusside (SN) and the effects of intracerebroventricular injection of physostigmine (PH) or hemicholinium-3 (HC-3) on the reflex changes in heart rate (HR) were observed.

PH (10 μg) increased basal mean arterial pressure (MAP) by 35%, reduced basal HR by 21%, enhanced the NE-induced bradycardia by 73%, and reduced the SN-induced tachycardia by 63%. These effects were rapid in onset and lasted about 30 min. HC-3 (20 μg) did not modify MAP but reduced HR by 31% and decreased both the NE-induced bradycardia (by 50%) and the SN-induced tachycardia (by 45%). The HC-3 bradycardic effect started within minutes and lasted for about 1 h, while its depressor effect on the reflexes began only after 15 min and continued for several hours. Neither PH nor HC-3 modified the pressor effect of NE, the depressor effect of SN or the responsiveness of peripheral muscarinic receptors.

These results suggest that a central nervous cholinergic mechanism modulates baroreceptor reflexes.

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The contractile effects of 5-hydroxytryptamine on the rat isolated vas deferens

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Contractions of the rat vas deferens produced by 5-hydroxytryptamine (5-HT) have been reported by Nishino, Irikura & Takayanagi (1970), Ozawa & Katsuragi (1974) and Miranda (1976). We have extended these observations to bisected vasa deferentia and with the use of receptor antagonists.

Vasa deferentia from Wistar rats (250 to 450 g body weight) were suspended in Krebs–Henseleit solution ($Na^+ = 144$, $K^+ = 5.8$, $Ca^{2+} = 2.5$, $Mg^{2+} = 1.2$, $HCO_3^- = 25$, $H_2PO_4^- = 1.2$, $SO_4^{2-} = 1.2$, $Cl^- = 129$, glucose = 11.1 mM/l) at 36 to 37°C and contractions were recorded isometrically.

5-HT (50 μg/ml) usually produced a phasic contraction that reached a maximum in 24 to 60 s and then declined towards the baseline. Rhythmic contractions, usually starting at the peak of this contraction, continued until wash-out (up to 3 h). There was considerable variation in the sensitivity of different tissues. After transverse bisection of the vas (Pennefather, Vardolov & Heath, 1974; Anton, Duncan & McGrath, 1977) the initial phasic response was largely confined to the epididymal half. The rhythmic contractions were also much greater in the epididymal half, and in some cases the prostatic half showed no response of any type (Table 1).

Table 1 Contractions produced by 5-HT (50 μg/ml) in rat bisected vasa deferentia ($n = 8$)

	Epididymal half	*Prostatic half*
Initial phasic: tension (g)	0.44 ± 0.06	0.07 ± 0.02
Rhythmic contractions:		
Tension (g)	0.52 ± 0.09	0.26 ± 0.06
Frequency (per min)	4.1 ± 0.4	1.8 ± 0.4

Methysergide hydrogen maleate (0.01 to 1 μg/ml) always reduced and in some cases abolished the rhythmic contractions produced by 5-HT. In these concentrations, methysergide did not affect the response to methoxamine (2 μg/ml). In nine out of thirteen experiments, phentolamine HCl (0.1 to 1 μg/ml) markedly reduced the response to 5-HT, but in the remaining four it had little effect. After either phentolamine or methysergide, any residual activity was usually abolished by the other antagonist. After chronic guanethidine treatment to destroy the adrenergic nerves (Heath, Evans, Gannon, Burnstock & James,

1972) the initial phasic part of the 5-HT response was augmented ($P < 0.001$). The rhythmic contractions were generally also more frequent and larger, though this was not statistically significant.

After chronic guanethidine treatment, the phasic response was blocked by methysergide, and the rhythmic contractions were reduced or abolished by methysergide or by methysergide plus phentolamine.

Nishino *et al.* (1970) found that the 5-HT response in the rat vas deferens was blocked by reserpinisation, by phentolamine and by imipramine and they concluded that 5-HT was acting by the release of endogenous noradrenaline. Our results are partly in agreement with theirs, but in addition we have found that the response is still present after chronic guanethidine treatment and that in some preparations it is not blocked by phentolamine, but is always antagonised by methysergide. We therefore conclude that part of the response to 5-HT is mediated by serotonin receptors on the smooth muscle.

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References

ANTON, P.G., DUNCAN, M.E. & MCGRATH, J.C. (1977). Analysis of the anatomical basis for the mechanical response to motor nerve stimulation of the rat vas deferens. *J. Physiol. Lond.*, **273**, 23–43.

HEATH, J.W., EVANS, B.K., GANNON, B.J., BURNSTOCK, G. & JAMES, V.B. (1972). Degeneration of adrenergic neurons following guanethidine treatment: an ultrastructural study. *Virchows Arch. Abt. B Zellpath.*, **11**, 182–197.

MIRANDA, H. (1976). Vas deferens desensitization by noradrenaline and other drugs. *Archs int. Pharmacodyn. Ther.*, **221**, 223–234.

NISHINO, K., IRIKURA, T. & TAKAYANAGI, I. (1970). Mode of action of 5-hydroxytryptamine on rat isolated vas deferens. *Nature Lond.*, **228**, 564–565.

OZAWA, H. & KATSURAGI, T. (1974). Ouabain-induced potentiation on the contractions of the guinea-pig vas deferens. *Eur. J. Pharmac.*, **25**, 147–154.

PENNEFATHER, J.N., VARDOLOV, L. & HEATH, P. (1974). Regional variation in the response of the rat vas deferens to field stimulation, to noradrenaline and to tyramine. *Clin. exp. Pharmac. Physiol.*, **1**, 451–462.

Post-junctional location of histamine receptors in mouse and rabbit vasa deferentia

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Histamine inhibits the twitch response to electrical stimulation of the mouse isolated vas deferens via a histamine H_2-receptor, and potentiates the twitch response in the rabbit isolated vas deferens via an H_1-receptor (Marshall, 1978; Bhalla & Marshall, 1980a, b). We now have investigated whether the histamine receptors are located pre- or post-junctionally.

Vasa deferentia were removed from T.O. mice (30 to 40 g) or rabbits (2.5 to 3.0 kg) and suspended between parallel platinum electrodes in oxygenated magnesium-free Krebs solution at 37°C. Responses were recorded isometrically with a Grass FT.03 transducer.

In the mouse vas deferens concentration-dependent contractions were obtained with acetylcholine (EC_{50}, 50% effective concentration, 13.0 ± 1.8 μM, mean ± s.e. mean) and phenylephrine (EC_{50}, 13.0 ± 1.3 μM). Acetylcholine contractions were competitively antagonised by atropine (10 nM) and those to phenylephrine by phentolamine (100 nM). Histamine (100 to 3.0 mM) and dimaprit (300 to 3.0 mM), the selective histamine H_2-receptor agonist (Parsons, Owen, Ganellin & Durant, 1977) reduced submaximal contractions produced by acetylcholine (30 μM) and phenylephrine (30 μM) (maximum reduction, 55 ± 7% and 87 ± 2%, respectively). The selective histamine H_2-receptor antagonist cimetidine (30 μM) (Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975) antagonised the effect of both histamine and dimaprit.

To investigate whether the histamine inhibition was dependent on intact sympathetic nerve terminals mice were pretreated (at 48 h and 24 h prior to removal of their vasa) with 6-hydroxydopamine (1 mmol/kg, i.v., Jones & Spriggs, 1975). The vasa did not respond to electrical stimulation (0.2 Hz, 2.0 ms, 64 V). Contractions of the vas were again obtained to phenylephrine (EC_{50}, 2.0 ± 0.3 μM). Histamine (EC_{50}, 0.79 ± 0.09 μM) reduced a submaximal contraction produced by phenylephrine (3.0 μM) and this reduction was antagonised by cimetidine (30 μM).

In the mouse the effect of histamine (3, 30 and 300 μM) on the release of l-[7,8-^{3}H]-noradrenaline from four vasa (40 min incubation with 590 nM, s.p. 28.3 Ci/mmol, Amersham) was measured. Histamine did not alter either the spontaneous efflux of [^{3}H]-norad-

renaline or the stimulation-induced (1.0 Hz, 120 s, 2.0 ms pulse width) increase in [^{3}H]-noradrenaline overflow ($P > 0.05$).

In the rabbit vas deferens phenylephrine produced concentration-dependent contractures (EC_{50}, 16.5 ± 2.5 μM) which were antagonised by phentolamine (100 nM). Histamine (3.0 to 300 μM) potentiated submaximal contractures to phenylephrine (10 μM) and this potentiation was antagonised by mepyramine (10 nM).

We conclude that the histamine (H_2) receptor in the mouse vas deferens is mainly situated post-junctionally because (i) histamine and dimaprit reduce contractions produced by acetylcholine and phenylephrine, (ii) the effect of histamine persists in vasa pretreated with 6-hydroxydopamine, and (iii) histamine does not reduce the stimulated overflow of [^{3}H]-noradrenaline. In rabbit vasa the results are also consistent with a post-junctional location of the histamine (H_1) receptor.

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References

BHALLA, P. & MARSHALL, I. (1980a). Histamine H_2-receptor antagonists in the mouse isolated vas deferens. *Br. J. Pharmac.*, **68**, 149P.

BHALLA, P. & MARSHALL, I. (1980b). Histamine H_1- and H_2-receptor mediated effects in vasa deferentia from the rat, guinea-pig and rabbit. *Br. J. Pharmac.* In Press.

BRIMBLECOMBE, R.W., DUNCAN, W.A.M., DURANT, G.J., EMMET, J.C., GANELLIN, C.R. & PARSONS, M.E. (1975). Cimetidine—a non-thiourea H_2-antagonist. *J. Int. Med. Res.*, **3**, 86–92.

JONES, M.E.L. & SPRIGGS, T.L.B. (1975). Noradrenaline and motor transmission in the vas deferens of the mouse. *Br. J. Pharmac.*, **53**, 323–331.

MARSHALL, I. (1978). An inhibitory histamine H_2-receptor in the mouse vas deferens. *Br. J. Pharmac.*, **62**, 447P.

PARSONS, M.E., OWEN, D.A.A., GANELLIN, C.R. & DURANT, G.J. (1977). Dimaprit—[S-[3-(N,N-dimethylamino)-propyl]isothiourea]—a highly specific histamine H_2-receptor agonist. Part 1, Pharmacology. *Agents and Actions*, **7**, 31–37.

The effects of indapamide on the responses to electrical stimulation of *in vitro* preparations from the rat

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Previous studies have shown that indapamide [chloro-4-N-(methyl-2-indolinyl-1)-sulphamoyl-3-benzamide] is an active antihypertensive agent in man (Seedat & Reddy, 1974) and animals (Finch, Hicks & Moore, 1977).

A prolonged antihypertensive action of indapamide, associated with impairment of sympathetic nerve function had been indicated in rats (Hicks, 1979). While reduced vascular reactivity to both electrical stimulation and vasoactive agonists had been demonstrated *in vivo* after sub-chronic treatment with indapamide (Finch, Hicks & Moore, 1977; Hicks, 1979), a reduced responsiveness to agonists has only been demonstrated at high concentrations *in vitro* (Finch & Hicks, 1978).

A further analysis of the acute effects of indapamide on responses in *in vitro* preparations of the rat has therefore been made. Male Sprague–Dawley (CFE) rats (200 to 300 g) were used for all experiments, $n = 6$ to 8.

Isolated preparations (vas deferens, 1 g tension; portal vein, 0.5 g tension; and phrenic nerve diaphragm), were incubated in Krebs at 37°C and gassed with 95% O_2 and 5% CO_2. Anococcygeus muscle (0.5 g tension) was superfused with Krebs (3 ml/min). Contractions were induced either by transmural stimulation (anococcygeus, 20 V, 0.4 ms, 0.1 to 10 Hz; portal vein, 20 V, 1 ms, 1 to 50 Hz; vas deferens, 20 V, 0.1 ms, 1 to 25 Hz), or by noradrenaline (NA) over the range 10^{-8} to 5×10^{-5} g/ml, and recorded isometrically. Responses to two doses of tyramine (5×10^{-6} and 10^{-5} g) were obtained in the anococcygeus preparation. Twitch responses in hemidiaphragm preparations were elicited by stimulation of the phrenic nerve (40 V, 0.1 ms, 0.25 Hz). Left and right kidneys were cannulated through the aorta and renal arteries under pentobarbitone (60 mg/kg i.p.) anaesthesia. The isolated kidneys were perfused at constant flow (2 ml/min) with filtered Krebs, and vasoconstriction in renal vascular beds was measured as an increase in back perfusion pressure, using a Bell and Howell pressure transducer (L2221) attached to a side arm of the cannula (Collis & Van Houtte, 1977).

All recordings were made using Devices M2 Chart recorders.

In the superfused anococcygeus muscle preparation, indapamide (5×10^{-5} and 10^{-4} M, 1 h) effected a dose-dependent reduction of the contractions elicited by low frequency stimulation ($P < 0.001$). A significant ($P < 0.05$) antagonism of the response to tyramine (10^{-5} g) was effected by indapamide at a concentration of 10^{-4} M. Antagonism of the responses to transmural stimulation in the portal vein was only achieved with concentrations of indapamide 10^{-4} and 5×10^{-4} M ($P < 0.05$). In the rat vas deferens only the second phase of contraction was inhibited by indapamide (10^{-4} M) at low frequencies of stimulation (3 to 12 Hz); the initial twitch response remained unaffected.

Indapamide (10^{-4} M) did not antagonize the responses induced by NA in any of these three preparations, neither was there any antagonism of the twitch response of the diaphragm during constant stimulation of the phrenic nerve by this concentration of indapamide. This indicates that the skeletal muscle innervation of this preparation was resistant to the effects of indapamide.

In the rat isolated kidney, the vasoconstriction elicited by periarterial stimulation was significantly reduced ($P < 0.05$) by indapamide (10^{-5} and 10^{-4} M) in a dose-dependent manner. At a concentration of 10^{-4} M, indapamide also antagonized the responses to NA ($P < 0.05$) in this preparation.

In all preparations only partial reversal of antagonized responses was effected by washing the preparations in normal Krebs for 60 min.

In conclusion, indapamide proved an effective antagonist of responses induced by electrical stimulation in smooth muscle preparations of the rat *in vitro*, but was inactive in a skeletal muscle preparation. There was some antagonism of postsynaptic-receptor mediated responses to NA, but only at high concentrations, and not in every preparation where the effects of electrical stimulation were reduced. The reasons for this divergence of effect are not clear.

References

COLLIS, M. & VAN HOUTTE, P.M. (1977). Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats. *Circ. Res.*, **41(6)**, 759–767.

FINCH, L., HICKS, P.E. & MOORE, R.A. (1977). Changes in vascular reactivity in experimental hypertensive animals following treatment with indapamide. *J. Pharm. Pharmac.*, **29**, 739–743.

FINCH, L. & HICKS, P.E. (1978). Effects of indapamide on blood pressure and cardiovascular reactivity in experimental hypertensive animals. *Seventh Int. Congress Pharmac.*, 2698.

HICKS, P.E. (1979). The effects of long term oral treatment with indapamide on the development of DOCA-salt hypertension in rats: vascular reactivity studies. *Clin. Exp. Hypertension.*, **1(6)**, 713–731.

SEEDAT, Y.K. & REDDY, J. (1974). Clinical evaluation of SE 1520 in the treatment of hypertension. *Clin. Ther. Res.*, **16**, 275–280.

Inhibition of noradrenaline uptake by N-methyl-benzylamine

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In a previous paper (Pirisino, Ciottoli, Buffoni, Anselmi & Curradi, 1979) N-methyl-benzylamine (NMB) was identified in human urine and plasma after the administration of pargyline. This observation raised our interest in the possible pharmacological effect of this pargyline metabolite.

In some preliminary experiments we observed a potentiation of the effect of noradrenaline (NE) on mouse and rat vas deferens.

Vasa deferentia from male Wistar rats were used with the method described by Anton, Duncan & McGrath (1977). The experiments were carried out in Krebs-Henseleit medium gassed with 95% O_2 and 5% CO_2. In this condition, after 10 min of preincubation, NMB increased NE-induced contractions of the isolated vas deferens. ED_{50} of NE (1.23 ± 0.04 μg.ml^{-1}) was decreased to 0.52 ± 0.16 μg/ml by NMB 7 nM and to 0.3 ± 0.04 μg/ml by NMB 35 nM (mean ± s.e. mean of six experiments each).

NMB also decreases [^{3}H]-NE uptake which was studied in rat according to the method of Iversen & Langer (1969). The effect of NMB on [^{3}H]-NE

Table 1 Per cent inhibition of [^{3}H]-NE uptake in rat isolated vas deferens by NMB and cocaine

	10^{-4} M	10^{-5} M	10^{-6} M
NMB	85.0 ± 2.4	59.8 ± 0.3	19.6 ± 6.0
Cocaine	72.6 ± 5.5	42.6 ± 4.1	15.7 ± 5.7

Results are the mean of five experiments ± s.e. mean.

uptake was compared with that of cocaine. The results are reported in Table 1.

The potentiation of NE in isolated vas deferens may be explained by inhibition of NE uptake.

References

ANTON, P.G., DUNCAN, M.E. & MCGRATH, J.C. (1977). An inhibition of post-ganglionic motor transmission in the mammalian vas deferens by D-Lysergic acid diethylamide. *J. Physiol.*, **231**, 251–270.

IVERSEN, L.L. & LANGER, S.Z. (1969). Effects of phenoxybenzamine on the uptake and metabolism of noradrenaline in the rat heart and vas deferens. *Br. J. Pharmac.*, **37**, 627–637.

PIRISINO, R., CIOTTOLI, G.B., BUFFONI, F., ANSELMI, B. & CURRADI, C. (1979). N-methyl-benzylamine a metabolite of pargyline in man. *Br. J. Clin. Pharmac.*, **7**, 595–598.

Local (iris, vein) pharmacological tests in morphine addiction

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Two drops of a 4% morphine hydrochloride solution instilled into the conjunctival sac, provoked a long-lasting miosis (evaluated by electronic pupillometry) in the treated, but not in the untreated eye of healthy volunteers. Two drops of a 0.16% naloxone solution, instilled into the morphine pre-treated eye, promptly reversed the miosis.

Furthermore, in an experiment with six healthy volunteers, conjunctival naloxone was unable to reverse the miosis, induced by a single 4 mg parenteral dose of morphine; whereas in a volunteer addict, the pupil of the right eye, treated with conjunctival naloxone, dilated promptly and durably. The mydriatic action of naloxone eye drops was verified in the same addict by repeating the instillation on different days, in the left and then again in the right eyes.

The sensitivity to 5-hydroxytryptamine creatinine sulfate (5-HT) of the smooth muscle in hand dorsal veins was evaluated using the computerized venotest (CVT) in 18 male subjects (6 morphine or heroin addicts, 12 healthy volunteers). The venoconstrictive threshold dose was 1 to 10 ng and 250 to 500 ng of 5-HT, respectively in addicts, tested during initial abstinence, and in controls; the venospasm was expressed in venoconstrictor units. In addicts a 10 mg bolus of morphine sulfate, injected into a vein contralateral to the one tested, promptly reduced the 5-HT sensitivity to the levels of controls. Naloxone (0.4 mg) administered into the tested vein, during the morphine reducing effect on the 5-HT venospasm, induced a prompt, intense and lasting venospasm together with the clinical signs of abstinence. In a male volunteer, not included in the two subject groups, who was declared from the urine to be an addict (even though morphine was absent and naloxone test was negative) a venospasm was observed with a normal venocostrictive threshold dose (500 ng). The striking 5-HT hyper-responsiveness of venous smooth muscle in addicts is in agreement with the similar (even if of smaller proportions) phenomenon detected during the 'silent' abstinence after a short (3 days) and slight (30 mg/day) parenteral morphine treatment in volunteers (Sicuteri, Del Bianco & Anselmi, 1979) as well as with the 5-HT hypersensitivity of ileum in morphine-tolerant guinea-pigs (Schulz & Goldstein, 1973).

In conclusion, the conjunctival naloxone test (simple and inexpensive) and CVT (expensive and sophisticated) may be suggested, to be if confirmed, two 'local' tests devoid of general reactions for the diagnosis of addiction.

References

SICUTERI, F., DEL BIANCO, P.L. & ANSELMI, B. (1979). Morphine abstinence and serotonin supersensivivity in man: analogies with the mechanism of migraine? *Psychopharmacology*, **65**, 205–209.

SCHULZ, R. & GOLDSTEIN, A. (1973). Morphine tolerance and supersensitivity to 5-hydroxytryptamine in the myenteric plexus of the guinea-pig. *Nature*, **244**, 168–170.

Structure-activity relationships in histamine H_1 receptor antagonists

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In two previous papers (Bertolasi, Borea, Gilli & Sacerdoti, 1980a, b) we have reported the crystal and molecular structures of two histamine H_1 receptor antagonists, carbinoxamine maleate and cyclizine hydrochloride, and have shown that their conformations in the crystal correspond reasonably well to those calculated by minimizing the non-bonded intramolecular potential energy. The preferred conformation of antihistamines assumes particular importance in consequence of the high degree of conformational freedom of these molecules and in view of the hypothesis (Witiak, Muhi-Eldeen, Mahishi, Sethi & Gerald, 1971; James & Williams, 1974) that a fixed distance between the amino nitrogen and the centroid of an unsaturated ring is determinant for antihistaminic activity.

In the present study, after comparison of the crystal structures of 15 histamine H_1 receptor antagonists of known crystal structure, we checked the correspondence between the conformations observed in the solid state and those calculated minimizing the non-bonded intramolecular potential energy, in a trial of picking out the geometrical features, if any, common to all molecules under investigation. Moreover, we wanted to test, on a wide experimental basis, the hypothesis whether histamine H_1 receptor antagonists mimic, from a structural point of view, the agonist parent molecule histamine.

The preliminary results of this study indicate that: (1) all the molecules under examination crystallize in a conformation which corresponds reasonably well to a minimum of the intramolecular energy of the free monocation, the discrepancies between calculated and observed values being ascribable to rough deficiencies in the calculation model more than to the effects of the crystal field; (2) all the antihistamines under examination are similar when projected on the plane defined by the protonated amino nitrogen and the centres of gravity of the two unsaturated rings; (3) all histamine H_1 receptor antagonists under examination have an unsaturated 5- or 6-membered ring whose centre of gravity lies at a distance of 6.00 to 6.40 Å from the amino nitrogen and therefore do not mimic histamine (in which the corresponding distance in the fully extended conformation is about 4.90 Å).

In conclusion, although drug activity is determined by many factors, such as lipophilicity, pK_a, steric hindrance of substituents etc., making it difficult to establish a close correlation between stereochemical factors and biological activity, the present results support the idea of a stereochemical vector of antihistaminic activity which parallels the pharmacodinamic moiety identified, on the ground of chemical connectivity, by comparison of different histamine H_1 receptor antagonists (Witiak *et al.*, 1971).

References

BERTOLASI, V., BOREA, P.A., GILLI, G. & SACERDOTI, M. (1980a). Crystallographic and conformational studies on histamine H_1 receptor antagonists—I. Molecular structure of carbinoxamine maleate. *Acta Cryst.* In press.

BERTOLASI, V., BOREA, P.A., GILLI, G. & SACERDOTI, M. (1980b). Crystallographic and conformational studies on histamine H_1 receptor antagonists—II. Molecular structure of cyclizine hydrochloride. *Acta Cryst.* In press.

JAMES, M.N.G. & WILLIAMS, G.J.B. (1974). The crystal and molecular structure of the antihistaminic drug triprolidine hydrochloride monohydrate. *Can. J. Chem.*, **52**, 1880–1888.

WITIAK, D.T., MUHI-ELDEEN, Z., MAHISHI, N., SETHI, O.P. & GERALD, M.C. (1971). L(S)- and D(R)-3-amino-1-phenylpyrrolidines. Selective antagonists for histamine and acetylcholine receptors *in vitro*. *J. Med. Chem.*, **14**, 24–30.

Effects of Ca^{2+} withdrawal and verapamil on excitation–contraction coupling in sensitized guinea-pig airway smooth muscle

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Ovalbumin-sensitized guinea-pigs when exposed to aerosolized solutions of the protein develop a severe respiratory anaphylaxis that is a consequence of histamine released from airway mast cells. In this study we have examined excitation–contraction coupling in normal and sensitized tracheae with a view to characterizing the Ca^{2+} sources utilized in the histamine-induced contractions.

Spirally cut or single open-ringed preparations of tracheae taken from both normal and ovalbumin-sensitized guinea-pigs were suspended in Krebs–Henseleit solution (KHS) at 37°C and gassed with 5%

CO_2 in O_2. Histamine (4×10^{-7} M to 1×10^{-4} M) elicited concentration-dependent contractions. There was no significant difference between the concentration-effect curves to histamine in tracheae from normal and sensitized animals.

At low agonist concentrations loosely-bound Ca^{2+} play the principal role in contraction whereas high-affinity bound Ca^{2+} are more important at high agonist concentrations (Farley & Miles, 1978). For our Ca^{2+} efflux experiments we, therefore, selected concentrations of histamine that produced approximately 25% (1×10^{-6} M) and 90% (2.5×10^{-5} M) of the maximal response. Incubation of the tracheae in Ca^{2+}-free KHS for periods ranging from 10 min up to 180 min caused time-dependent reductions in the magnitude of contractions elicited by both concentrations of histamine. In each case the graphs of tension loss against time in Ca-free KHS were biphasic. In normal tracheae EC_{90} histamine contractions declined to about 10% of the control response after 60 min in Ca^{2+}-free KHS. Longer incubations produced little further reductions in tension. In contrast sensitized tissues attained the same 10% level after only 30 min. Both normal and sensitized tracheae contracted using EC_{25} histamine exhibited tension loss patterns similar to that observed with the high histamine concentration in sensitized tissues. Replotting these data in the form of a Ca^{2+}-efflux curve (Hurwitz & Joiner, 1970) revealed that in each case the decline in tension could be described by two exponentials; a fast component and a slow component. The respective half times of these components are:

EC_{90} normal 9.3 and 69.3 min; sensitized 3.4 and 13.9 min. EC_{25} normal 5.3 and 26.9 min; sensitized 3.4 and 18.6 min.

Verapamil (1×10^{-5} M to 1×10^{-3} M) caused a concentration-dependent inhibition of histamine-induced contractions. This antagonism was non-competitive in nature. There was no significant difference between the effects of verapamil on normal and sensitized tracheae.

It is concluded, therefore, that whilst there is no alteration in either the affinity or the intrinsic activity of histamine in sensitized tracheae there is an obvious change in the utilization and binding affinities of the activator calcium. Such alterations are most evident at high histamine concentrations. Furthermore, the results with verapamil taken together with the shift in emphasis from high- to low-affinity bound Ca^{2+} sources in sensitized tracheae indicate that in this smooth muscle tissue the activator Ca^{2+} may be principally extracellular in origin.

References

FARLEY, J.M. & MILES, P.R. (1978). The sources of calcium for acetylcholine-induced contractions of dog tracheal smooth muscle. *J. Pharmac. Exp. Ther.*, **207**, 340–346.

HURWITZ, L. & JOINER, P.D. (1970). Mobilization of cellular calcium for contraction in intestinal smooth muscle. *Am. J. Physiol.*, **218**, 12–19.

An *in vitro* and *in vivo* comparison of the effects of cholinoceptor blockade on cat and baboon urinary bladders

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Human and baboon urinary bladders are very sensitive to cholinoceptor blockade *in vivo* (Brindley & Craggs, 1975) whereas the bladders of many other species including the cat are known to be relatively resistant. Unexpectedly responses of human and monkey bladder strips evoked by transmural electrical stimulation *in vitro* are only partially antagonized by atropine (Johns & Paton, 1977; Hindmarsh, Idowu, Yeates & Zar, 1977).

Experiments were performed to compare the effects of hyoscine on responses of baboon and cat bladders to sacral ventral root stimulation and of excized detrusor strips to transmural stimulation. Electrical stimulation was at 15 200 μs pulses/s for 15 s and at a supramaximal current. The responses evoked by transmural stimulation were blocked by tetrodotoxin (5×10^{-7} M) and were studied in the presence of guanethidine (4×10^{-5} M).

Responses of baboon bladders ($n = 8$) to sacral stimulation were very sensitive to hyoscine, 0.1 mg/kg i.v. reducing peak intravesical pressure by approx. 90%, the response fatiguing before the end of stimulation. In contrast responses of bladder strips to transmural stimulation were much less sensitive to hyoscine. Approximately the same concentration of hyoscine (0.23 μM, equivalent to 0.1 mg/kg assuming even distribution) reduced peak tension by only 10% and the tension at the end of stimulation by 54%. Even a high concentration of hyoscine (100 μM) reduced peak tension by only 21%.

Unlike the baboon, responses of cat bladders to sacral stimulation ($n = 5$) and of bladder strips to

transmural stimulation were similar. Hyoscine (10 mg/kg i.v. or 23 μM) reduced peak intravesical pressure by 36% and peak tension by 41%; the responses at the end of stimulation were reduced by 75% and 43%, respectively.

These results show that cat and baboon detrusor muscle *in vitro* is relatively insensitive to hyoscine irrespective of its sensitivity *in vivo*. The reasons why primate bladder should lose its sensitivity *in vitro* is of interest because low sensitivity of non-primate bladders has been attributed to non-cholinergic neuromuscular transmission (Taira, 1972). This explanation is unlikely for primates in view of their sensitivity *in vivo*.

References

BRINDLEY, G.S. & CRAGGS, M.D. (1975). The effect of atropine on the urinary bladder of the baboon and of man. *J. Physiol.*, **256**, 55P.

HINDMARSH, J.R., IDOWU, O.A., YEATES, W.K. & ZAR, M.A. (1977). Pharmacology of electrically evoked contractions of human bladder. *Br. J. Pharmac.*, **61**, 115P.

JOHNS, A. & PATON, D.M. (1977). Effect of indomethacin on atropine-resistant transmission in rabbit and monkey urinary bladder: evidence for involvement of prostaglandin in transmission. *Prostaglandins*, **13**, 245–254.

TAIRA, N. (1972). The autonomic pharmacology of the bladder. *Ann. Rev. Pharmacol.*, **12**, 197–208.

β_2 Adrenoceptor agonists inhibit gastric acid secretion in Shay rats

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The role and importance of the sympathetic nervous system in the physiology of gastric secretion are still controversial questions (Sanders, 1976). It has been reported that β_1 and β_2 adrenoceptor agonists were able to inhibit pentagastrin induced acid secretion in the dog (Daly, Long & Stables, 1978).

In Shay rats, three β_2 adrenoceptor agonists salbutamol, terbutaline and fenoterol have been assessed in order to evaluate their effects on total gastric secretion, total acidity and acid output, following the method previously employed by del Tacca, Soldani, Bernardini, Pierini & Giomini (1978). Significant inhi-

Table 1 Effects of beta adrenoceptor agonists on total gastric secretion (ml/3 h ± s.e. mean), total acidity (mEq/l ± s.e. mean), and acid output (mEq/3 h ± s.e. mean) in Shay rats (Sprague–Dawley strain, body weight 250 g)

	Controls saline 0.9% i.p. (n = *12*)	*Salbutamol 1 mg/kg i.p.* (n = *12*)		*Salbutamol + Sotalol (1 mg/kg i.p.) (30 mg/kg i.p.)* (n = *12*)
Total gastric secretion	4.10 ± 0.45	1.67 ± 0.16	$P < 0.001$	3.36 ± 0.35 N.S.
Total acidity	73.40 ± 8.89	40.16 ± 5.10	$P < 0.005$	79.60 ± 10.2 N.S.
Acid output	0.301 ± 0.03	0.067 ± 0.005	$P < 0.001$	0.275 ± 0.05 N.S.
	Controls saline 0.9% i.p. (n = *10*)	*Terbutaline 1 mg/kg i.p.* (n = *10*)		*Terbutaline + Sotalol (1 mg/kg i.p.) (30 mg/kg i.p.)* (n = *10*)
Total gastric secretion	3.90 ± 0.40	1.50 ± 0.12	$P < 0.001$	4.25 ± 0.41 N.S.
Total acidity	66.80 ± 7.31	37.18 ± 4.80	$P < 0.005$	68.50 ± 7.21 N.S.
Acid output	0.290 ± 0.04	0.050 ± 0.006	$P < 0.001$	0.318 ± 0.04 N.S.
	Controls saline 0.9% i.p. (n = *10*)	*Fenoterol 1 mg/kg i.p.* (n = *10*)		*Fenoterol + Sotalol (1 mg/kg i.p.) (30 mg/kg i.p.)* (n = *10*)
Total gastric secretion	4.15 ± 0.48	1.63 ± 0.11	$P < 0.001$	3.26 ± 0.41 N.S.
Total acidity	79.00 ± 9.21	35.58 ± 7.50	$P < 0.005$	66.95 ± 4.76 N.S.
Acid output	0.273 ± 0.03	0.058 ± 0.004	$P < 0.001$	0.218 ± 0.07 N.S.

Drugs dissolved in saline were injected in volume of 0.25 ml.
n = number of experiments. Significance of differences between controls and treated animals was calculated by using Student's *t* test.

bition was obtained of total gastric secretion, total acidity and acid output for all three drugs examined (Table 1). Mucosal blood flow, measured by amidopyrine clearance, appeared not to be significantly affected. The β blocker sotalol failed to modify either gastric secretion or mucosal blood flow, but prevented the gastric inhibitory action of β_2 adrenoceptor agonists. In addition to this β_1 selective adrenoceptor agonist prenalterol (1 mg/kg i.p.) did not modify gastric secretion (12 expts).

These results suggest that gastric secretion inhibition by β_2 agonists was not due to the reduction of mucosal blood flow, but to the activation of β_2 adrenoceptors which might decrease gastric secretion by inhibiting histamine formation or release (Lundell & Svensson, 1974). The clinical use of β_2 agonists in asthmatic patients suffering from gastric lesions might be indicated.

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References

DALY, M.J., LONG, J.M. & STABLES, R. (1978). The role of β_1 and β_2 adrenoceptors in the inhibition of gastric acid secretion in the dog. *Br. J. Pharmac.*, **64**, 153–157.

DEL TACCA, M., SOLDANI, G., BERNARDINI, C., PIERINI, P. & GIOMINI, M.L. (1978). The inhibitory action of ketamine on the rat's gastric secretion. *Arch. int. Pharmacodyn.*, **231**, 308–316.

LUNDELL, L. & SVENSSON, S.E. (1974). Implications of gastric mucosal histamine in inhibition by isoprenaline of pentagastrin induced gastric secretion. *Br. J. Pharmac.*, **52**, 69–75.

SANDERS, D.J. (1976). A review. The agents and actions of sympathetic nerve and catecholamine inhibition of gastric mucosal function. *Agents and Actions*, **6**, 385–388.

11-deoxy-13,14-didehydro-16S-methyl-PGE$_2$ (K11550), a new antiulcer agent

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We assessed the potency of 11-deoxy-13,14-didehydro-16S-methyl-PGE$_2$ (K11550) in inhibiting gastric secretion, stress-induced gastric lesions and in producing enteropooling activity.

Gastric acid secretion inhibition was tested in pylorus ligated rats (Shay, Sun & Gruenstein, 1954) (160 $\pm$ 10 g) according to Franceschini, Mizzotti, Ceserani, Mandelli & Usardi (1977). ED_{50} for PGE$_2$ and K11550 administered s.c. were 159 μg/kg (fiducial limits, $P = 0.95$: 144 to 175) and 65 μg/kg (55 to 75). ED_{50} for K11550 after oral administration was 3607 μg/kg (3208 to 4117) while PGE$_2$ is inactive.

PGE$_2$ and K11550 injected s.c. immediately before stress (Usardi, Franceschini, Mandelli, Daturi & Mizzotti, 1974) to rats (130 $\pm$ 10 g) fasted for 16 h but given free access to water, gave ED_{50} of 150 μg/kg (140 to 161) and 20.7 μg/kg (19.9 to 21.5) for protection from gastric lesions. Orally administered PGE$_2$ and K11550 protected gastric mucosa at ED_{50} of 484 μg/kg (444 to 527) and 182 μg/kg (167 to 198). Gastric mucosa lesions were scored 1 h after stress according to Osterloh, Lagler, Staemmler & Helm (1971). Enteropooling was studied in rats (170 $\pm$ 10 g) according to Franceschini *et al.*, 1977.

Oral ED_{25} for K11550 was 407 μg/kg (378 to 440); after s.c. injection the ED_{25} was 455 μg/kg (419 to 497).

We also compared K11550 with 16,16-dimethyl-PGE$_2$, a potent antiulcer compound (Robert, Schultz, Nezamis & Lancaster, 1976), on stress ulcers and on enteropooling.

This compound was more potent than K11550 in inhibiting stress-induced gastric lesions: the ED_{50}s were 14.1 μg/kg (12.6 to 15.8) and 18.9 μg/kg (16.3 to 22.1) after s.c. and oral administration respectively. It was also a powerful enteropooling agent with ED_{25} = 38.3 μg/kg (34.6 to 42.7) injected s.c. and 7.45 μg/kg (7.20 to 7.73) after oral administration. PGE$_2$, K11550 and 16,16-dimethyl-PGE$_2$ were administered s.c. in 0.2 ml/100 g body weight and orally in 0.5 ml/100 g body weight. At least 12 male ICEM:CER (SPF Caw) rats were used for each dose.

Since K11550 had much less enteropooling effect than 16,16-dimethyl-PGE$_2$ and is chemically more stable, it might prove useful in the management of gastric ulcers, despite its weaker antiulcer activity.

References

FRANCESCHINI, J., MIZZOTTI, B., CESERANI, R., MANDELLI, V. & USARDI, M.M. (1977). Structure–activity relationship of some acetylenic-PGE$_2$ derivatives. *Pharmacol. Res. Commun.*, **9**, 899–908.

OSTERLOH, VON G., LAGLER, F., STAEMMLER, M. & HELM, F. (1971). Pharmakologische und toxicologische Untersuchungen über Benzilsäure-(N,N-dimethyl-2-hydroxymethyl-piperidinum)-ester-methyl-sulfat)-ein neues Spasmolyticum. *Arzneimittel-Forschung*, **16**, 901–910.

ROBERT, A., SCHULTZ, J.R., NEZAMIS, J.E. & LANCASTER, C. (1976). Gastric antisecretory and anti-ulcer properties of PGE$_2$, 15-methyl PGE$_2$, and 16,16-dimethyl PGE$_2$: intravenous, oral and intrajejunal administration. *Gastroenterology*, **70**, 359–370.

SHAY, H., SUN, D.C.H. & GRUENSTEIN, M. (1954). A quantitative method for measuring spontaneous gastric secretion in rat. *Gastroenterology*, **26**, 906–913.

USARDI, M.M., FRANCESCHINI, J., MANDELLI, V., DATURI, S. & MIZZOTTI, B. (1974). Prostaglandin VIII: a proposed role for PGE_2 in the genesis of stress-induced gastric ulcers. *Prostaglandins*, **8**, 43–51.

Changes in [^{3}H]-clonidine binding after short-term surgical denervation of rat submaxillary gland

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The subclassification of α-adrenoceptors into α_1 and α_2 categories (Langer, 1974) is based on pharmacological differences and not on anatomical localization (Langer, Briley & Dubocovich, 1980). Surgical sympathetic denervation or chemical denervation with 6-hydroxydopamine have been used extensively in attempts to establish the pre- or postsynaptic localization of α_2-adrenoceptors (Langer *et al.*, 1980).

In most studies carried out in the central nervous system chronic chemical denervation with 6-hydroxydopamine either increased or failed to decrease the specific binding of the selective α_2-adrenoceptor agonist, [^{3}H]-clonidine (U'Pritchard & Snyder, 1979; U'Prichard, Bechtel, Rouot & Snyder, 1979).

We report here studies of changes in [^{3}H]-clonidine binding during the first 24 and 48 h after surgical denervation of the rat salivary gland, since at this time degeneration of noradrenergic nerve endings occurs but the postsynaptic component of supersensitivity is not yet developed.

Unilateral postganglionic sympathetic denervation of the rat submaxillary gland was achieved by excision of the superior cervical ganglion. The submaxillary gland membranes were prepared by homogenization and centrifugation and the resulting preparation resuspended in Tris/HCl 50 mM pH 7.5. [^{3}H]-clonidine binding to α_2-adrenoceptors and [^{3}H]-QNB binding to muscarinic cholinoceptors was measured by the filtration technique. Specific binding was defined as the binding inhibited in the presence of 10 μM phentolamine ([^{3}H]-clonidine) or 10 μM atropine ([^{3}H]-QNB) and represented 60 to 70% of the total binding at 2.3 nM [^{3}H]-clonidine and 70 to 80% at 1 nM [^{3}H]-QNB.

Right and left glands from unoperated rats showed similar [^{3}H]-clonidine binding characteristics. Twenty four h after surgical sympathetic denervation, the apparent dissociation affinity constant (K_d) was decreased approx. 3-fold [innervated left gland: $K_d = 2.7 \pm 0.2$ nM ($n = 5$); denervated right gland: $K_d = 0.8 \pm 0.1$ nM ($n = 5$) ($P < 0.001$)]. The maximal binding (B_{max}) was increased by about 60% [innervated left gland: $B_{max} = 22.9 \pm 1.4$ fmol/mg protein ($n = 5$); denervated right gland: $B_{max} = 37.4 \pm 1.8$ fmol/mg protein ($n = 5$) ($P < 0.001$)]. Changes of approximately the same magnitude were also observed 48 h after the operation.

These modifications in the apparent dissociation affinity constant and number of sites were specific to the α_2-adrenoceptors. The binding of [^{3}H]-QNB to muscarinic cholinoceptors showed no alteration between innervated and denervated glands over the same time period following denervation.

The lack of decrease in [^{3}H]-clonidine binding after surgical denervation supports the view that a large proportion of the sites in this tissue are situated postsynaptically. The increase in the number and affinity of the [^{3}H]-clonidine binding sites after only 24 and 48 h of denervation suggests that the presence of the noradrenergic nerve ending may modulate the number and properties of the postsynaptic α_2-adrenoceptors. Either the neurotransmitter itself or another substance in noradrenergic nerve terminals with trophic actions may be responsible for the transsynaptic modulation of the α_2-adrenoceptor population.

Preliminary experiments in which surgical denervation was carried out after the depletion of the noradrenaline stores by reserpine showed similar changes in [^{3}H]-clonidine binding suggesting that the postulated trophic factor is probably not noradrenaline itself.

The early increase in [^{3}H]-clonidine binding described here complicates the interpretation of experiments using sympathetic denervation to study the pre- or postsynaptic localization of α_2-adrenoceptors. Until the nature and time-course of these early changes in binding are clarified the conclusions of the pre- or postsynaptic localisation of the α_2-adrenoceptors based on denervation experiments will remain questionable.

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References

LANGER, S.Z. (1974). Presynaptic regulation of catecholamine release. *Biochem. Pharmacol.*, **23**, 1793–1800.

LANGER, S.Z., BRILEY, M.S. & DUBOCOVICH, M.L. (1980). Adrenergic receptor mechanisms in the central nervous system in relation to catecholamine neurons. In *Central Adrenaline Neurons: Basic Aspects and their Role in Cardiovascular Functions* (Wenner-Gren Symposium). eds Fuxe, K., Goldstein, M., Hökfelt, B. Oxford: Pergamon Press (in press).

U'PRICHARD, D.C. & SNYDER, S.H. (1979). Distinct α-noradrenergic receptors differentiated by binding and physiological relationships. *Life Sci.*, **24**, 79–88.

U'PRICHARD, D.C., BECHTEL, W.P., ROUOT, B.M. & SNYDER, S.H. (1979). Multiple apparent α-noradrenergic receptor binding sites in rat brain: effect of 6-hydroxydopamine. *Molec. Pharmacol.*, **16**, 47–60.

Effects of emulsifiers on the elimination of intravenously-administered fat emulsions measured by gamma scintigraphy

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Oil-in-water emulsions of certain vegetable oils such as soybean oil stabilised with egg lecithin are widely used in parenteral nutrition. Egg lecithin is a mixture of phospholipids, the principle components being phosphatidylcholine (PC) and phosphatidylethanolamine (PE) with minor components such as lysolecithin (LPC). Geyer (1967) and Jeppsson & Rossner (1975) have both shown that the clearance of fat emulsions from the circulation is dependent on the nature of the emulsifier.

In vitro studies with mouse peritoneal macrophage and *Acantamoeba Castellini-Neff* have shown differences in the uptake of fat emulsion droplets stabilised by purified egg lecithin (PC + PE), purified egg lecithin with lysolecithin (PC + PE + LPC) or polyoxyethylene–polyoxypropylene co-polymers (Pluronics) (Hansrani, 1980).

The elimination of [^{123}I]-labelled soybean oil emulsions stabilised with these emulsifying agents has been followed in the rabbit using the technique of gamma scintigraphy. [^{123}I]-Labelled soybean oil (specific activity 100 μCi/g oil) was prepared by the method of Lubran & Pearson (1958). 10% v/v [^{123}I]-soybean oil in water was emulsified with (i) 1.2% w/v PC + PE (4:1); (ii) 1.2% w/v PC + PE (4:1) + 0.25% w/v LPC; (iii) 1% w/v Pluronic F68. The particle size of all emulsions was less than 1 μM as determined by Coulter counter analysis.

Three groups, each of four rabbits received 6 ml of the emulsion by intravenous administration through a marginal ear vein. Scintigrams were taken for 13 to 24 h after dosing and the amount of [^{123}I] determined in whole body, neck, thorax, liver/stomach and bladder regions as described by Hardy, Kellaway, Rogers & Wilson (1980).

The preparations were rapidly taken up in the liver/stomach region. A biphasic elimination pattern was noted for the group of rabbits dosed with PC + PE stabilised fat emulsion, the slower exponential phase had a half-life of 12 ± 1.4 h (mean ± s.d.). The stomach half-life of [^{131}I]-sodium iodide in the rabbit is 13 h (Hardy, Leeson & Wilson, 1978) which suggests that the slower elimination phase represents stomach turnover of [^{123}I].

Addition of lysolecithin to the PC + PE caused a more rapid disappearance of the fat emulsion from the circulation with a much slower elimination from the liver with a mean half-life of 207 ± 1.2 h. Emulsion stabilised with Pluronic F68 had a complex initial phase and then was cleared at the same rate as the PC + PE stabilised with a mean elimination half-life 14.5 ± 1.2 h.

These results concur with evidence obtained in the *in vitro* experiments described earlier and indicate that the nature of the emulsifying agent may influence the rate of elimination from uptake sites *in vivo*.

References

GEYER. (1967). Studies on the metabolism of intravenous fat emulsions. *Fette. Med.* **6**, 59–61.

HANSRANI, P. (1980). Ph.D. Thesis, University of Nottingham

HARDY, J.G., LEESON, M. & WILSON, C.G. (1978). The use of the gamma camera to measure the disposition and fate of iodide in the rabbit. *Br. J. Pharmac.*, **64**, 468–469P.

HARDY, J.G., KELLAWAY, I.W., ROGERS, J. & WILSON, C.G. (1980). Distribution and fate in the rabbit of liposomes containing [^{131}I]-sodium iodide. *J. Pharm. Pharmacol.* (in press).

JEPPSSON, R. & ROSSNER, S. (1975). The influence of emulsifying agents and of lipid-soluble drugs on the fractional removal rate of lipid emulsions from the blood stream of the rabbit. *Acta. pharmacol. et. toxicol.*, **37**, 134–144.

LUBRAN, M. & PEARSON, J.D. (1958). A screening test for steatorhoea using [^{131}I]-labelled triolein. *J. Clin. Path.*, **11**, 165–169.

Long-term feeding of rats with semi-synthetic diets substituted in protein components: effect on kidney and liver

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Renal calcification and impaired liver function are commonly found in experimental animals especially when they are fed on semisynthetic diets containing casein or soy protein isolates. We wanted to investigate whether the above mentioned phenomena could be obtained also with diets containing proteins derived from bovine plasma. Sprague–Dawley rats of both sexes were fed for 6 months on semi-synthetic diet containing as the only source of protein, casein or bovine plasma proteins. We investigated liver and kidney fine morphology and liver microsomal enzyme activity at different times of feeding. After 8 weeks, rats given semi-synthetic diets had histological signs of nephrocalcinosis. The severity of the histological lesions increased proportionally with the time of feeding reaching its maximum level after 6 months.

Liver microsomal enzyme activity was normal after 2 months, while it was markedly increased after 6 months. At this time mitochondrial and endoplasmic reticulum modifications were also present in liver cells. Liver and kidneys changes were more severe in female rats. Replacing, after 2 months, the semi-synthetic diet with an open formula diet, the renal lesions remained unchanged at least for a further 2 months. The relevance of these data will be discussed in relationship with the toxicc logical evaluation of the use of non-conventional protein in the diet.

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Less TCDD persists in liver 2 weeks after a single dose to mice fed chow with added charcoal or cholic acid

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Interest in the extremely toxic compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in relation to allied occupational disease occurring in several countries including the U.K. (May, 1973) was recently renewed because of environmental pollution, the latest, most dramatic case still causing concern in Italy (Manara & Garattini, 1978). The complex mechanism by which TCDD given to laboratory animals produces a peculiarly delayed type of toxicity involving multiple organ systems and functions is far from clear, but a striking and presumably related feature is the unusually large fraction of the administered dose that accumulates and persists in the liver (Vinopal & Casida, 1973).

Thus we considered means of shortening the persistence of TCDD in the body with a view to reducing associated toxic actions. Male C57Bl/6J mice, 18 to 21 g, maintained under controlled environmental conditions and fed standard powdered chow ('Altromin R', A. Rieper, Italy) were given [^{3}H]-TCDD (Kor Isotopes, U.S.A., S.A. 51 Ci/mM, 90% radiochemically pure in our test) dissolved in acetone: corn oil, 1:6, 5 ml/kg either by stomach tube (p.o.) or i.p.

Animals were then randomly allocated either to a control group (continuing on standard chow) or to one of the other groups which were switched to food with the test substances added as indicated in the Table. All mice were killed 14 days after receiving TCDD and their livers were frozen on dry ice and stored at −25°C until processed (1:15, weight: volume, acetone homogenates radioassayed by liquid scintillation).

As found by Vinopal & Casida (1973), livers contained a substantial fraction of the administered dose, and TLC chromatography of occasional samples (solvents, n-hexane:ethyl ether, 16:1; $R_F = 0.80$) gave no evidence of TCDD biotransformation in any group. However radioactivity (Table 1) from TCDD in the livers of mice fed either charcoal or cholic acid, or both, was well below control values (as low as 36%). We are at present unable to explain how chow with these additives affects the persistence of TCDD in the liver, but the results of additional experiments lead us to believe that the underlying mechanism is close to that involved in prevention of toxicity. No deaths occurred, in fact, in mice fed charcoal ($n = 16$) and cholic acid ($n = 11$) within 60 days of TCDD (90 μg/kg) given respectively p.o. and i.p., whereas 7/15 and 9/20 of the appropriate controls died during this interval.

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Table 1 Radioactivity in liver of mice fed chow with different additives 14 days after a single dose of [^{3}H]-TCDD

Experiment	*A*	*B*	*C*
Standard chow	17.3 ± 3.2 (6)	19.2 ± 1.6 (6)	10.3 ± 0.8 (5)
5% Vegetable charcoal	6.3 ± 1.1** (6)	15.3 ± 2.6** (7)	5.6 ± 0.8** (5)
0.5% Cholic acid	13.1 ± 2.8* (6)	—	—
5% Animal charcoal	—	13.8 ± 1.3** (7)	—
5% Vegetable charcoal plus 1% Cholic acid	—	10.9 ± 2.4** (7)	—
5% Animal charcoal plus 1% Cholic acid	—	9.1 ± 1.1** (7)	—
4% Cholestyramine	14.5 ± 1.7 (6)	—	—

The fractions of the administered dose recovered in liver are given as percentages, means ± s.d. (*n*). TCDD doses were 7.6, p.o.; 11.0, i.p.; and 12.5 p.o. μg/kg. for experiments A, B and C respectively. Controls received standard chow throughout the experiment; chows with added test substances were started right after TCDD administration (experiment A) or 3 days later (experiments B and C).
** $P < 0.01$; * $P < 0.05$ (Duncan's New Multiple test); — not investigated.

References

MANARA, L., & GARATTINI, S. (1978). Seveso: premature optimism. *Nature Lond.*, **276**, 556.

MAY, G. (1973). Chloracne from the accidental production of tetrachlorodibenzodioxin. *Br. J. Ind. Med.*, **30**, 276–283.

VINOPAL, J.H. & CASIDA, J.E. (1973). Metabolic stability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mammalian liver microsomal systems and in living mice. *Arch. Environ. Contam. Toxicol.*, **1**, 122–132.

Dexamethasone inhibition of carrageenan paw oedema in the rat requires *de novo* synthesis of putrescine

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The anti-inflammatory effects of dexamethasone have recently been shown to be mediated by polypeptide(s) synthesized following binding of the steroid to the glucocorticoid receptor and induction of gene expression (Tsurufuji, Sugio & Takemasa, 1979). Biosynthesis of the polyamines putrescine, spermidine and spermine is intimately interrelated with the synthesis of nucleic acids and proteins (Tabor & Tabor, 1976). We have explored the role of the polyamines in the manifestation of the anti-inflammatory effects of dexamethasone using α-difluoromethylornithine (DFMO) (Metcalf, Bey, Danzin, Jung, Casara & Vevert, 1978), an irreversible inhibitor of ornithine decarboxylase (ODC). ODC catalyses the formation of putrescine from ornithine and provides the only biosynthetic route in animal tissues for putrescine and spermidine (Tabor & Tabor, 1976).

Male Sprague–Dawley rats weighing 180 to 230 g supplied by Charles River, France, were used. Carrageenan paw oedema was induced by injection of 0.1 ml of 1.0% (w/v) carrageenan (Copenhagen Pectin Factory) in normal saline into the subplantar region of the foot. Foot diameters were measured at intervals thereafter for 3 h.

Dexamethasone (Soludecadron, Merck), 0.25 to 0.5 mg/kg injected s.c. 3 h before carrageenan, inhibited oedema formation by 75 to 80%. The effects of dexamethasone were inhibited dose-dependently to a maximum of 50% by DFMO (50, 100, 200 or 500 mg/kg) given i.p. 1 h before the steroid. The effect was selective in that the anti-inflammatory effect of indomethacin (10 mg/kg i.p.), was unaltered following treatment with DFMO (500 mg/kg).

Pretreatment with aminoguanidine sulphate (Eastman), an inhibitor of putrescine catabolism (25 mg/kg) i.p., 16 h before DFMO completely prevented blockade of the anti-inflammatory effect of dexamethasone. Aminoguanidine itself had no effect on carrageenan oedema. Reversal of the anti-inflammatory effect of dexamethasone was also obtained with 5-hexyne-1,4-diamine (50 mg/kg, RMI 71696) an irreversible inhibitor of ODC some four times more potent than DFMO *in vivo* (Seiler, Danzin, Prakash & Koch-Weser, 1978).

One site where the polyamines might be involved in the mechanism of action of dexamethasone is the liver. Dexamethasone (0.25 mg/kg) induced rises in hepatic ODC activity and putrescine concentration which were maximal at 4 h. Spermidine and spermine concentrations were unchanged. The increases in ODC activity and putrescine were completely prevented by DFMO (200 mg/kg) injected 1 h before the steroid.

Thus, compounds which irreversibly inhibit ODC reduce the anti-inflammatory effects of dexamethasone, but not indomethacin, on carrageenan paw oedema in the rat. Since the effect is prevented by an inhibitor of putrescine catabolism, the data suggest *de novo* synthesis of putrescine is an essential factor in the induction of new protein synthesis known to be involved in the anti-inflammatory action of dexamethasone (Tsurufuji *et al.*, 1979).

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References

METCALF, B.W., BEY, P., DANZIN, C., JUNG, M.J., CASARA, P. & VEVERT, J.P. (1978). Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogues. *J. Am. Chem. Soc.*, **100**, 2551–2553.

SEILER, N., DANZIN, C., PRAKASH, N.J. & KOCH-WESER, J. (1978). Effects of ornithine decarboxylase inhibitors *in vivo*. In *Enzyme-Activated Irreversible Inhibitors*. ed. Seiler, N., Jung, M.J. and Koch-Weser, J. pp. 55–71. Amsterdam: Elsevier/North Holland Biomedical Press.

TABOR, C.W. & TABOR, H. (1976). 1,4-diaminobutane (putrescine), spermidine, and spermine. *A. Rev. Biochem.* **45**, 285–306.

TSURUFUJI, S., SUGIO, K. & TAKEMASA, F. (1979). The role of glucocorticoid receptor and gene expression in the anti-inflammatory action of dexamethasone. *Nature (Lond.)*, **280**, 408–410.

The prostaglandin synthesis inhibitor in placental homogenates is located in trapped blood elements

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We recently reported that the microsomal supernatant from rat placental homogenates contained a proteinaceous factor which inhibited arachidonic acid metabolism (Harrowing & Williams, 1979). The placenta is richly vascularized and blood plasma has been reported to contain a prostaglandin (PG) synthetase inhibitor (Saeed, McDonald-Gibson, Cuthbert, Capas, Schneider, Gardiner, Butt & Collier, 1977). We have therefore carried out experiments to determine whether the PG synthetase inhibitor in placental homogenates is due to trapped blood elements.

Day 22 pregnant rats were killed and the placentae, livers, spleens and brains removed. 25% (w/v) homogenates of tissues were prepared in distilled water. Preparation of the microsomal supernatant, removal of endogenous arachidonic acid and lyophylization were carried out (Harrowing & Williams, 1979). The ability of these extracts to inhibit conversion of radiolabelled arachidonic acid by decidual microsomes from the rat pregnant uterus was investigated as described previously (Williams & Downing, 1977). The inhibitory activities of these tissues (ID_{50} values) were found to be placenta 2.95 ± 0.62 mg (mean $\pm$ s.e. mean, $n = 4$); liver 1.13 ± 0.2 mg; spleen 2.80 ± 0.91 mg. Thus all the tissues which contained large amounts of trapped blood elements inhibited PG synthesis whereas brain tissue produced no detectable inhibition.

In separate experiments pregnant rats were anaesthetized with pentobarbitone sodium (70 mg/kg i.p.). The uteri and placentae were then perfused via the

dorsal aorta with Kreb's solution until visibly free of blood; the liver was perfused via the hepatic portal vein. The tissues were then treated as described above. Perfusion removed all inhibitory activity from placental microsomal supernatants but only marginally reduced activity from the liver. Thus, in the placenta all PG synthetase inhibition is attributable to trapped blood elements. This was confirmed by showing that microsomal supernatants from fractions of rat blood contained potent PG synthetase inhibitory activity; whole blood ($ID_{50} = 1.57 \pm 0.06$); plasma ($ID_{50} = 0.7 \pm 0.07$ mg) and also the erythrocytes ($ID_{50} = 0.65 \pm 0.08$ mg). Activity was still detectable in serum. Ultrafiltration of the plasma revealed that the majority of inhibitory activity had a mol. wt. >300,000. However, the possibility that more than one factor in whole blood was responsible for inhibition of PG synthesis could not be excluded as treatment of lyophylized red cell preparations with 30% (w/v) ammonium sulphate completely precipitated the inhibitory factor(s) but only 70% of activity in the plasma was precipitated.

These studies indicate that trapped blood elements in tissues could adversely affect PG synthesis. Blood elements could also account for the inhibitory activity reported in microsomal supernatants by many workers.

P.D.H. is an S.R.C. (CASE) Student.

References

HARROWING, P.G. & WILLIAMS, K.I. (1979). Homogenates of rat placenta contain a factor(s) which inhibits uterine arachidonic acid metabolism. *Br. J. Pharmac.*, **67**, 482 P.

SAEED, S.A., MCDONALD-GIBSON, W.J., CUTHBERT, J.L., SCHNEIDER, C., GARDINER, P.J. BUTT, N.M. & COLLIER, H.O.J. (1977). Endogenous inhibitor of prostaglandin synthetase. *Nature (Lond.)*, **70**, 32–36.

WILLIAMS, K.I. & DOWNING, I. (1977). Production by rat decidual microsomes. *Prostaglandins*, **14**, 813–817.

In vivo N-dealkylation of chloropromazine and chlorimipramine: a single-dose kinetic study in the rat

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It has been shown recently in this laboratory that chronic treatment of rat with chlorimipramine (CI) produced an accumulation of phospholipids which was particularly evident in the lungs. In addition, both CI and its demethylated metabolite (DMCI) were preferentially stored in this organ (Della Corte, Gremigni, Megazzini, Mobilio & Sgaragli, 1979). Chlorpromazine (CPZ) also preferentially accumulates in the lungs (Wechsler & Roizin, 1960) where, however, it does not seem to induce the morphological changes typical of drug-induced lipidosis (Lüllmann-Rauch & Scheid, 1975). In the search for a biochemical mechanism involved in the development of CI-induced lipidosis, we compared the N-dealkylation of CI and CPZ in the rat.

Male Sprague–Dawley rats 200 to 250 g body wt. were orally treated with a single dose (90 mg/kg

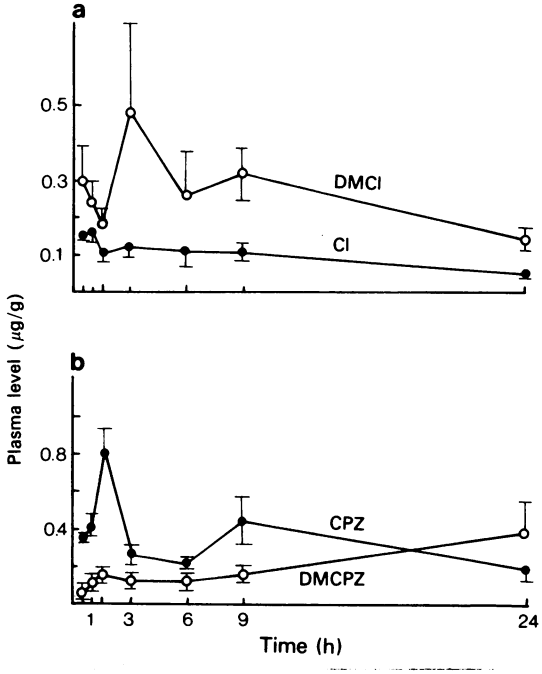

Figure 1 Plasma levels of chlorimipramine (CI), desmethylchlorimipramine (DMCI), chlorpromazine (CPZ) and desmethylchlorpromazine (DMCPZ) following oral administration of CI or CPZ in a single dose of 90 mg/kg body wt. Each point represents the mean value of five rats ± s.e. mean.

body wt.) of CI.HCl (Ciba-Geigy, Milano, Italy) or CPZ.HCl (Sigma Chem. Co., Saint Louis, Missouri, U.S.A.). Tissue levels (plasma, erythrocytes, brain, liver, lung, heart, kidney, spleen and peritoneal fat) of CI and DMCI, CPZ and DMCPZ at different times following drug administration were measured by gas liquid chromatography using a nitrogen detector. As shown in Figure 1, the plasma levels of CI + DMCI (a) and of CPZ + DMCPZ (b) reached similar values during the overall period of observation. However, while DMCI was always higher than CI, DMCPZ levels were lower, reaching CPZ values only at 24 h. In addition, DMCI and CPZ levels exhibited a clear periodicity. All compounds markedly accumulated in the lung. DMCPZ, however, in contrast with its distribution in the other tissues, was found in higher concentrations already 1 h after treatment. These findings suggest that in the rat CI underogoes a more rapid N-demethylation than CPZ. Furthermore, DMCPZ seems to be preferentially accumulated inside the lung when compared with CPZ. A marked capacity of the lung to N-dealkylate tertiary amine compounds could explain this behaviour.

References

DELLA CORTE L., GREMIGNI, D. MEGAZZINI, I., MOBILIO, R. & SGARAGLI, G.P. (1979). Chlorimipramine-induced phospholipidosis: biochemical and pharmacokinetic observations. *Br. J. Pharmac.*, **67**, 449–450P.

LÜLLMANN-RAUCH, R. & SCHEID, D. (1975). Intraalveolar foam cells associated with lipidosis-like alterations in lung and liver of rats treated with tricyclic psychotropic drugs. *Virchow's Arch. B Cell Path.*, **19**, 255–268.

WECHSLER, M.B. & ROIZIN, L. (1960). Tissue levels of chlorpromazine in experimental animals. *J. Ment. Sci.*, **106**, 1501–1505.

Biliary excretion of β-lactamines in rats: *in vitro* and *in vivo* determination

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Ampicillin is useful for the treatment of biliary tract infections: the amount of the excreted drug in the bile is sufficient to have an antibacterial activity (Neuman, 1973; Brogard, Pinget, Meyer, Dorner & Lavillaureix, 1977).

We have compared in rats the biliary excretion of ampicillin and its related compounds (amoxycillin, bacampicillin) and other β-lactamines, such as carbenicillin, sulbenicillin, cefazolin and cefazedon (a new cephalosporin with high drug-protein binding). For this purpose two different methods have been utilized: (a) *in vitro* perfusion (3 h) of the rat isolated liver (Kvetina & Guaitani, 1969). The antibiotics were added to the perfusing medium in a concentration of 50 μg/ml; (b) *in vivo* drainage of common biliary duct (3 h) in the rat anaesthetized with urethane (1.5 g/kg i.p.). Antibiotics were given orally and parenterally (50 mg/kg).

Female Sprague–Dawley rats (200 to 250 g) were used. The bile was collected every 30 min. Antibiotic activity was determined in the bile and in the homogenized liver, using an agar plate diffusion method with Nutrient Agar Medium and *Staphylococcus aureus* 209 as test organism.

In Table 1 are reported the half hour maximum biliary excretion of the drugs, percentage of antibiotic excreted in the bile and present in the liver at the end of the experiments. At the end of the experiments, *in vitro* and *in vivo*, antibiotic therapeutic concentrations were still detectable in the bile.

Both these methods are valuable to show variations between antibiotics, even those which are chemically closely related. The concentration of antibiotics in the bile is strictly related to their physicochemical properties, to protein binding, metabolism, liver blood flow and mechanisms of transport (Levine, 1978). Moreover the *in vitro* system suggests a greater degree of hepatic inactivation for ampicillin and amoxycillin than that of other drugs.

References

BROGARD, J.M., PINGET, M., MEYER, C., DORNER, M. & LAVILLAUREIX, J. (1977). Biliary excretion of ampicillin: experimental and clinical study. *Chemotherapy (Basel)*, **23**, 213–226.

KVETINA, J. & GUAITANI, A. (1969). A versatile method for the *in vitro* perfusion of isolated organs of rats and mice with particular reference to liver. *Pharmacology*, **2**, 65–81.

LEVINE, W.G. (1978). Biliary excretion of drugs and other xenobiotics. *Ann. Rev. Pharmacol. Toxicol.*, **18**, 81–96.

NEUMAN, M. (1973). Aspects pratiques et intérêt de l'étude du passage biliaire des antibiotiques chez l'homme. *Lille méd.*, **3**, 319–324.

Table 1 Biliary excretion of β-lactamines *in vitro* and *in vivo* (3 h). In brackets number of animals

	In vitro					*In vivo*				
	Amount	*Max. biliary excretion*		*Recovery %*		*50 mg/kg*	*Max. biliary excretion*		*Recovery %*	
Drug	*(mg)*	*Minutes*	*μg ($\bar{x}$ ± s.d.)*	*Bile*	*Liver*	*route*	*Minutes*	*μg ($\bar{x}$ ± s.d.)*	*Bile*	*Liver*
Ampicillin	2.75 (5)	30 to 60	61.5 ± 35.8	5.4	0.2	p.o. (6)	60 to 90	17.3 ± 7.7	0.9	0.3
Bacampicillin*	2.75 (5)	30 to 60	142.0 ± 29.2	22.7	4.3	p.o. (11)	60 to 90	125.1 ± 23.9	6.5	2.7
Amoxycillin	2.75 (6)	0 to 30	6.6 ± 2.6	1.1	0.6	p.o. (12)	0 to 30	23.3 ± 9.2	1.1	0.7
Carbenicillin	3.00 (4)	30 to 60	106.9 ± 9.2	14.1	4.0	i.m. (4)	90 to 120	75.7 ± 15.9	3.3	0.5
Sulbenicillin	3.00 (4)	30 to 60	266.4 ± 24.4	25.4	8.2	i.m. (4)	90 to 120	235.4 ± 56.6	10.2	3.7
Cefazedon†	2.75 (4)	0 to 30	155.0 ± 34.6	10.9	10.9	i.m. (4)	30 to 60	143.8 ± 54.8	4.2	1.3
Cefazolin†	2.75 (4)	0 to 30	51.0 ± 6.2	5.1	4.2	i.m. (4)	60 to 90	108.6 ± 16.6	2.8	1.1

* Bacampicillin is determined as ampicillin: 100 mg are equivalent to 74 mg.
† The duration of the experiments was 2 h.

An extremely sensitive new method for evaluating DNA damage induced by mutagens and carcinogens

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We used a very sensitive oscillating viscometer, with, above all, an extremely low shear strain ($<0.01 \times s^{-1}$). An oscillating viscometer of this type was first proposed for liquid metals by Gallina, Malvano & Omini (1971). The essential features and theory of this instrument were described in the same paper. It was adapted to the study of high molecular weight biopolymers for the first time by our group (Giaretti, Parodi & Brambilla, 1979).

It was clearly shown by Shooter (1976) that phosphodiester bridges, while extremely stable in alkali, are so large in number for a long DNA chain that their degradation rate is definitely not negligible (we can extrapolate ~2 breaks $\times 10^{-9}$ daltons $\times h^{-1}$, following 3 to 5 h of lag-time, at 20°C, 0.2 N NaOH). On the other hand, in a typical alkaline condition (pH 12.5, 22°C), the unwinding of the two strands is far from instantaneous (from our experiments it takes ~8 h to be ≥90% completed). The consequence of these two facts is that measurement of molecular weight of intact disentangled single stranded molecules is impossible, because when disentanglement is completed the molecular weight will be around 10^8 daltons. The only way to measure higher molecular weights is to measure disentanglement rates directly.

The oscillating viscometer is apparently very convenient for this type of measurement because a complete series of viscosity data can be collected continuously from the same sample. In this way, relative variabilities of apparent intrinsic viscosities at different times (on which accuracy of disentanglement rate measurements depend) are automatically avoided. At the beginning of the experiment the apparent intrinsic viscosity is very low (≥10% of maximum viscosity), and a maximum plateau level is slowly reached in about 8 h in control rat liver nuclei DNA. With both dimethyl sulphate *in vitro* (30 min incubation at 2°C, pH 7.3), and with N-diazoacetylglycine amide and dimethylnitrosamine *in vivo* the disentanglement rate was already significantly faster for doses that could be linearly extrapolated as equivalent to molecular weights between 2 and 5×10^{10} daltons (from previous alkaline sucrose gradient sedimentation and alkaline elution data). For doses equivalent to mol-

ecular weights of 1-2 × 10^9 daltons disentanglement was completed within only 30 minutes. By consequence, contrary to the hypothesis of Filippidis & Meneghini (1977), if single stranded DNA subunits do very rapidly develop in alkali with a molecular weight around 10^8 daltons, maximum plateau viscosity would have been reached immediately. Alkali-resistant non-deoxynucleotide linkers are not excluded.

We think that our method makes possible the evaluation of extremely small levels of DNA damage and, at the same time, disproves the idea of the existence in rat DNA of small independent subunits separable in alkali.

This investigation is supported by grants from C.N.R.—Progetto Finalizzato 'Controllo della Crescita Neoplastica'.

References

FILIPPIDIS, E. & MENEGHINI, R. (1977). Evidence for alkali-sensitive linkers in DNA of African green monkey kidney cells. *Nature (Lond)*, **269**, 445–447.

GALLINA, V., MALVANO, R. & OMINI, M. (1971). A new kind of oscillating crucible viscometer. *Rev. Sci. Instrum.*, **42**, 1607–1613.

GIARETTI, W., PARODI, S. & BRAMBILLA, G. (1979). Un nuovo viscosimetro per DNA nativo. *Atti V Congresso Nazionale Cibernetica e Biofisica*, Pisa, pp. 197–202.

SHOOTER, K. V. (1976). The kinetics of the alkaline hydrolysis of phosphotriesters in DNA. *Chem.-Biol. Interactions*, **13**, 151–163.

Role of agonists and antagonists of histamine lymphocyte receptors on functional expression of the immune system

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Histamine exerts inhibitory effects on lymphocyte functions mediated by an H_2 receptor (Askenase, 1977). On the other hand, *in vitro* and *in vivo* studies show that depletion of histamine receptor bearing cells enhances the immune response (Weinstein & Melmon, 1976) and that histamine activates suppressor T cells (Rocklin, Greineder & Melmon, 1979). Such cells can be activated *in vitro* after ConA stimulation (Shou, Schwartz & Good, 1976).

In order to better investigate the role of histamine and of antagonists specific for H_2 receptors on *in vitro* responses of human lymphocytes, Ficoll-Hypaque purified cells were incubated with histamine or cimetidine (SK&F) at different concentrations and subsequently checked for mitogenic response and suppressor activity.

Histamine treatment inhibited the mitogenic response to ConA and PHA (T cells), as well as to PWM and SpA (B cells) at different rates and drug concentrations (Table 1a), while cimetidine had no influence.

Since the highest histamine effect was obtained in the ConA system, the suppressor activity was measured after ConA treatment by pre-incubating lymphocytes with the drugs for 48 h, washing out and adding these cells to fresh drawn autologous lymphocytes. Both histamine and cimetidine did not alter the control levels of suppression in this system.

Table 1 Suppression of (a) mitogenic lymphocyte response by different histamine concentrations, and (b) lymphocyte response to ConA by different concentrations of ConA activated not retained lymphocytes from H-BSA-S or BSA-S columns

	Histamine concentration (M)		
(a) *Mitogen*	10^{-4}	10^{-3}	5×10^{-3}
PHA	82 ± 15	85 ± 15	63 ± 18
ConA	38 ± 22	47 ± 18	43 ± 18
PWM	90 ± 17	91 ± 9	75 ± 13
SpA	79 ± 15	86 ± 12	74 ± 19

	Cell concentration ($\times 10^{-3}$)				
(b) *Cells from*	3	6	12	25	50
H-BSA-S	0	0	0	7	21
BSA-S	3	6	6	31	65

3/5 cases showing similar results.

In addition, lymphocytes were passed through histamine–bovine serum albumin–sepharose (H–BSA–S) or bovine serum albumin–sepharose (BSA–S, control) columns to remove histamine receptor bearing cells and the suppressor activity was similarly evaluated on the non-retained cells. Such activity was reduced (Table 1b) after histamine-cell depletion, although with considerable variability for different donors and samples.

These data show that histamine produces inhibiting effects on PHA and more significantly on ConA re-response, while cimetidine does not. Both drugs did not alter the expression of suppressor activity on the ConA system. However, histamine columns partially deplete the suppressive cell population.

References

ASKENASE P.W. (1977). Role of basophils, mast cells, and vasoamines in hypersensitivity reactions with a delayed time course. *Prog. Allergy*, **23**, 199–320.

ROCKLIN R.E., GREINEDER D.K. & MELMON K.L. (1979). Histamine-induced suppressor factor (HSF): further studies on the nature of the stimulus and the cell which produces it. *Cell. Immunol.*, **44**, 404–415.

SHOU L., SCHWARTZ S.A. & GOOD R.A. (1976). Suppressor cell activity after Concanavalin A treatment of lymphocytes from normal donors. *J. Exp. Med.*, **143**, 1100–1110.

WEINSTEIN Y. & MELMON K.L. (1976). Control of immune response by cyclic AMP and lymphocytes that adhere to histamine columns. *Immun. Commun.*, **5**, 401–416.

Cadralazine and its identified metabolites: comparative effects on blood pressure and on arterial smooth muscle

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Cadralazine[1] ethyl 2-[6-[(2-hydroxypropyl)ethylamino]3-pyridazinyl]hydrazine carboxylate is a new antihypertensive drug arising from systematic studies on a series of hydrazino pyridazines.

Results from the screening (Parravicini, Scarpitta, Dorigotti & Pifferi, 1979) and pharmacodynamic evaluation (Pifferi, Parravicini, Scarpitta & Semeraro, 1978) showed that the drug is characterized by an antihypertensive effect gradual in onset and by a good oral absorption both in rats (SHR) and in dogs (Grollman). Further studies confirmed the above results and showed that cadralazine acts as a peripheral vasodilator. In fact, in awake normotensive dogs it reduced peripheral resistance and concomitantly increased heart rate, cardiac output, renal and iliac blood flow (Capri, Dorigotti & Semeraro, 1978). Furthermore the drug is devoid of blocking activity on ganglionic transmission, adrenergic neurons and α-adrenoreceptors and neither does it have a direct activity on arterial smooth muscle *in vitro*.

These findings may suggest that cadralazine produces the antihypertensive effect after metabolic activation. Metabolic studies (Simonotti, Zanol, Citerio & Pifferi, 1980) indicated that urine of orally treated

[1] Proposed generic name for ISF 2469.

Table 1 Activity data on cadralazine and metabolites I, II, III

	SHR rats *Hypotensive and tachycardic potency*							*Rabbit aortic strips relaxation*
cpd	*BP* ED_{25} *(mg/kg p.o.)*	*Peak effect (h)*	*HR* ED_{25} *(mg/kg p.o.)*	*BP* ED_{25} *(mg/kg i.v.)*	*Peak effect (h)*	*HR* ED_{25} *(mg/kg i.v.)*	*AR**	pD_2
Cadralazine	1.8	5	2.7	2.3	3	2.4	1.28	3
I	>30	—	>30	>10	—	>10	—	3
II	>30	—	>30	>10	—	>10	—	3
III	0.56	≤1	0.61	0.038	≤0.5	0.033	0.07	5.37

* AR = BP activity ratio ED_{25} i.v./ED_{25} p.o.

spontaneously hypertensive rats (SHR) contained a consistent amount of unchanged drug; two main metabolites, 3-oxotriazolo[4,3b]pyridazine (I) and 3-methyltriazolo[4,3b]pyridazine (II); and a low quantity of 3-hydrazino-6-[(2-hydroxypropyl)-ethylamino]pyridazine (III). So it was considered important to evaluate the activities of the compounds so far identified. Antihypertensive and smooth muscle relaxing effects were examined respectively on SHR after oral and i.v. administration and on rabbit aortic strips during adrenaline contraction (10^{-7} M) (Table 1).

Metabolites I and II were devoid of the studied activities, while metabolite III caused a dose dependent relaxation of arterial smooth muscle and its antihypertensive activity was confirmed (Pifferi, Parravicini, Carpi & Dorigotti, 1975) and found faster in onset and greater than that of cadralazine mostly after i.v. administration.

These results substantiate our previous assumption and indicate that cadralazine exerts its antihypertensive effect through a metabolic activation in which metabolite III seems to be directly involved.

References

CARPI, C., DORIGOTTI, L. & SEMERARO, C. (1978). Pharmacological profile of a new antihypertensive drug: ethyl 2-[6-[(2-hydroxypropyl)ethylamino]-3-pyridazinyl]hydrazine carboxylate. *7th International Congress of Pharmacology*, July 16 to 21, Paris Abs. 2700.

PARRAVICINI, F., SCARPITTA, G., DORIGOTTI, L. & PIFFERI, G. (1979). Derivati della 3-idrazino piridazina. *II Farmaco*, [Ed. Sc.] **34**, 299–310.

PIFFERI, G., PARRAVICINI, F., CARPI, C. & DORIGOTTI, L. (1975). Synthesis and antihypertensive properties of new 3-hydrazinopyridazine derivatives. *J. Med. Chem.*, **18**, 741–746.

PIFFERI, G., PARRAVICINI, F., SCARPITTA, G. & SEMERARO C. (1978). Synthesis and anti-hypertensive activity of new 6-substituted 3-alkyl and 3-acyl hydrazinopyridazine. *6th International Symposium on Medicinal Chemistry*, September 4 to 7, Brighton Abs. 17.

SIMONOTTI, L., ZANOL, M., CITERIO, L. & PIFFERI, G. (1980). Degradation and metabolic studies on cadralazine, a new antihypertensive agent. *7th International Symposium on Mass Spectrometry in Biochemistry, Medicine and Environmental Research*, Milan, June 16 to 18.

The effects of Cd^{2+} on the responsiveness of the rat isolated perfused kidney to periarterial stimulation, noradrenaline and potassium ions

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Cadmium (Cd^{2+}) has been shown to induce hypertension in rats following chronic or acute treatment (Perry, Erlanger & Perry, 1977; Fadloun & Leach 1980; Perry, Erlanger, Yunice & Perry, 1967). Chronic treatment of rats with Cd^{2+} potentiates the responsiveness of *in vivo* and *in vitro* preparations to electrical stimulation, noradrenaline (NA) and potassium ions (K^+) (Fadloun & Leach, 1980a). Since an important role for the kidney in blood pressure control is established (Guyton, Coleman, Scheel, Manning & Norman, 1972) and that kidney selectively accumulates Cd^{2+} (Shaikh & Lucis, 1972), the effect of Cd^{2+} on the responsiveness of isolated perfused kidney vasculatures of rat has been studied.

Male Sprague–Dawley rats (200 to 300 g) were anaesthetized (sodium pentobarbitone 60 mg/kg i.p.) and the kidney removed (Collis & Vanhoutte, 1977), transferred to a warming jacket and perfused with Kreb's solution (37°C, 2 ml/g). Cd^{2+} was added to the perfusion fluid and reduction in osmotic pressure caused by changes in ionic composition were adjusted by equivalent moles of sucrose. The effects of NA, periarterial nerve electrical stimulation and K^+ on the perfusion pressure were assessed. All experiments were commenced 45 mins after commencement of the perfusion. Periarterial stimulation provoked frequency-related increases in perfusion pressure which were reduced (12, 25 Hz 80%) or abolished (1 to 6 Hz) by phentolamine (5×10^{-6} M). NA (8 to 256×10^{-9} g) and K^+ (26, 52 μM) administered into the perfusion fluid (0.1 ml), increased the perfusion pressure in a dose-dependent manner; NA-induced responses were abolished by phentolamine (10^{-6} M), whilst responses to K^+ (26, 52 μM) were not significantly affected by phentolamine (5×10^{-6} M).

Cd^{2+} (1 to 5 μM) inhibited the responses to periarterial stimulation (1 to 25 Hz), responses to stimulation (1 to 6 Hz) were abolished and the response to 25 Hz was reduced by 80% with Cd^{2+} (5 μM). NA evoked responses were less affected by Cd^{2+}; the maximum attainable response was reduced 50% with Cd^{2+} (5 μM). The K^+-induced responses were inhibited by Cd^{2+}, the largest dose (52 μM) being most affected (45% reduction). The order of decreasing sen-

sitivity caused by Cd^{2+} was periarterial stimulation > K^+ > NA.

To investigate the role of external Ca^{2+} on the inhibitory action of Cd^{2+}, the effects of reduced Ca^{2+} (1.27, 0.64 mM) were tested. Low frequencies (1 to 6 Hz) and K^+ (52 μM) were the most affected by lowering Ca^{2+} concentrations, however, NA responses were reduced but to a lesser extent. When lowered Ca^{2+} (1.27 mM) and Cd^{2+} (1 μM) were used in conjunction, the inhibitory effects were synergestic for all three test systems including NA. The inhibitory effects of Ca^{2+} (1.27 mM) and Cd^{2+} (1 μM) appeared similar to those seen with Cd^{2+} (5 μM).

The results obtained in the isolated perfused kidney are in a close agreement with previous studies using vascular/non-vascular sympathetically innervated tissues (Fadloun & Leach, 1979, 1980b) with the difference that the vascular responsiveness of the kidney is far more sensitive to the inhibitory effect of Cd^{2+}, a fact which may relate to the high ability of the kidney to accumulate heavy metals.

References

COLLIS, M.G., & VANHOUTTE, P.M. (1977). Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats. *Circulation Res.* **41**, 759–767.

FADLOUN, Z. & LEACH, G.D.H. (1979). The effects of Cd^{2+} on the responsiveness of the rat acococcygeus muscle and vas deferens to electrical stimulation, noradrenaline, tyramine and K^+. *Br. J. Pharmac.*, **66**, 495P.

FADLOUN, Z. & LEACH, G.D.H. (1980). The effects of Cd^{2+} on the myogenic activity and the responsiveness of the rat portal vein to perimural stimulation, noradrenaline and potassium ions. *Br. J. Pharmac.*, **68**, 181–182P.

FADLOUN, Z. & LEACH, G.D.H. (1980). *In vivo* and *in vitro* measurement of changes in sympathetic nervous control of vascular and non-vascular tissues in rats chronically treated with Cd^{2+}. *Br. J. Pharmac.* (in press).

GUYTON, A.C., COLEMAN, T.G., COWLEY, A.W., SCHEEL, K.W., MANNING, R.D. & NORMAN, R.A. (1972). Arterial pressure regulation: overriding dominance of the kidneys in long-term regulation and in hypertension. *Am. J. Med.*, **52**, 584–594.

PERRY, H.M., JR., ERLANGER, M., YUNICE, A. & PERRY, E.F. (1967). Mechanism of the acute hypertensive effect of intra-arterial cadmium and mercury in anaesthetized rats. *J. Lab. Clin. Med.*, **70**, 963–972.

PERRY, H.M. JR., ERLANGER, M. & PERRY E.F. (1977). Elevated systolic pressure following chronic low-level cadmium feeding. *Am. J. Physiol.*, **232**, H114–H121.

SHAIKH, Z.A. & LUCIS, O.J. (1972). Cadmium and zinc binding in mammalian liver and kidneys. *Arch. Environ. Health* **24**, 419–432.

Further studies on the effects of diltiazem on the rabbit isolated heart perfused with an oxygenated or a hypoxic solution: correlation: between ultrastructural and mechanical effects

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We have reported that the profound increase in the resting tension produced by hypoxia in the rabbit isolated heart was attenuated by diltiazem. In addition, during the 30-min reoxygenation period following hypoxia, the recuperation of the contractile force of the hearts pretreated with diltiazem was significantly better than that of control preparations (Boudot & Cavero, 1979).

The aim of this study is to verify whether the cardiac myocyte ultrastructure was also protected by diltiazem.

Hearts from New Zealand (Hy/Cr) rabbits ($n = 2$ for each of the four groups of this study) were perfused using the Langendorff technique with Krebs-Henseleit solution (gassed with 95% O_2 + 5% CO_2) and electrically paced (137 pulses/min). Resting tension and contractile force were measured. After a suitable stabilization period, perfusion was continued for 30 min with either a normoxic or an hypoxic (95% N_2 + 5% CO_2) perfusate containing no drug or 10 μM diltiazem (Boudot & Cavero, 1979). At the end of this time, the myocardium was fixed by perfusing the preparation with cold phosphate buffer containing glutaraldehyde (2.5%). Small samples of left ventricular myocardium were taken and prepared for electronmicroscopy studies.

As previously reported, the increase in resting tension observed in the rabbit heart perfused with an hypoxic medium was reduced (approx. 50%) by diltiazem. Furthermore, diltiazem induced a negative inotropic action in control normoxic preparation and accelerated the decline of contractile force produced by hypoxia in control preparations (Boudot & Cavero, 1979).

The structure of myocytes from the rabbit myocardium perfused with an oxygenated solution containing diltiazem showed no abnormality. Interestingly, glycogen granules appeared to be more abundant in the tisssue from preparations perfused with diltiazem than in that from the control myocardium.

The myocyte from the heart exposed to hypoxia exhibited contracted sarcomeres and scalloped nuclear or sarcolemmal membranes. Mitochondria had a matrix slightly clearer than normal. The amount of glycogen in these cells was clearly reduced when compared to that of preparations perfused with drug free oxygenated solution. Diltiazem treatment prevented this depletion of glycogen stores and protected the cells from the hypoxia induced structural damage to such an extent that they were not very different from myocytes sampled from the normoxic heart.

Droplets of lipids were not detected in the myocardial tissues of this study.

These results suggest that diltiazem is able to prevent the cellular damage produced by hypoxia. It is reasonable to attribute such a protection to a better availability of energy required for cellular homeostasis. This might be achieved through the negative inotropic effect of diltiazem which leads to a reduced consumption of intracellular fuels during the critical initial period of exposure to hypoxia. However, in the anaesthetised dog in which a brief period of coronary occlusion was applied, diltiazem was reported to reverse the depression of mitochondrial respiration produced by ischaemia and this protection was attributed to haemodynamic and biochemical actions and not to a decrease in myocardial contractile force (Nagao, Matlib, Franklin, Millard & Schwartz, 1980).

References

BOUDOT, J.P. & CAVERO, I. (1979). Effects of diltiazem and verapamil on the mechanical performance of the rabbit myocardium perfused with an oxygenated and hypoxic medium. *Brit. J. Pharmac.*, **69**, 485P–486P.

NAGAO, T., MATLIB, M.A., FRANKLIN, D., MILLARD, R.W. & SCHWARTZ, A. (1980). Effects of diltiazem, a calcium antagonist, on regional myocardial function and mitochondria after brief coronary occlusion. *J. Mol. Cell. Cardiol.*, **12**, 29–43.

The presynaptic effects of isoprenaline, D600 and dantrolene in rat atria

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Dantrolene sodium has been reported to reduce depolarization-induced release of intracellular bound calcium (Desmedt & Hainaut, 1979). In the present study the effects of D600 (methoxyverapamil) and dantrolene sodium were investigated on the isoprenaline-induced facilitation of [^{3}H]-transmitter overflow in rat atria.

Isolated atria from adult Wistar rats were incubated with [^{3}H]-noradrenaline for 30 min. Each tissue was mounted between silver electrodes in an organ bath containing Locke's solution at 37°C, gassed with 95% O_2 and 5% CO_2. Field stimulation was carried out at 1 Hz (suprathreshold voltage 50 V, 0.2 ms pulse width) for periods of 5 min at intervals of 25 min; three periods of stimulation were applied during each experiment. The tritium overflowing into the bathing solution before, during and after nerve stimulation was measured by liquid scintillation counting.

The treatments used and experimental results obtained are outlined in Table 1. Results are expressed as the ratio of the fractional release

Table 1 Effects of isoprenaline (ISOP), propranolol (PROP), D600 and dantrolene sodium (DANT) on [^{3}H]-transmitter overflow elicited by nerve stimulation

Treatment	S_3/S_1†
Control	0.92 ± 0.05
ISOP	1.24 ± 0.09**
PROP	0.71 ± 0.06**
ISOP + PROP	0.81 ± 0.06
D600	0.53 ± 0.06**
ISOP + D600	0.97 ± 0.11
DANT	0.70 ± 0.09*
ISOP + DANT	1.30 ± 0.10

† See text. Values are presented as mean ± s.e. mean.
* Significantly different from control (* $P < 0.05$, ** $P < 0.01$, Students t test). The treatments within brackets were compared with one another and found to be significantly different ($P < 0.01$). ISOP was added 15 min before S_3, inhibitors were introduced 15 min before S_2 and were present for the remainder of the experiment.

obtained between the first and third stimulation periods (S_3/S_1). Basal efflux approximately doubled in the presence of D600 (20 μM) but was not altered by the other treatments. Isoprenaline (0.02 μM) caused a facilitation of evoked release which was abolished by propranolol (0.1 μM) at a dose which itself reduced evoked release. Both D600 (20 μM) and dantrolene (20 μM) inhibited release, however D600 was the more effective of the two. As can be seen in Table 1, the reductions obtained with these calcium ion antagonists were offset by isoprenaline.

The isoprenaline-induced facilitation of transmitter release and the antagonism of this effect by propranolol are indicative of the presence of presynaptic β-adrenoceptors in this tissue. It would appear that D600 has interfered with excitation–secretion coupling in a manner which did not antagonise the action of isoprenaline. It is therefore possible that these two agents affect intraneuronal free calcium levels by different mechanisms. The results obtained with dantrolene do not allow a distinction to be made between the nature of its inhibitory effect and that of D600.

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Reference

DESMEDT, J. & HAINAUT, K. (1979). Dantrolene and A23187 ionophore: specific action on calcium channels revealed by the aequorin method. *Biochem. Pharmac.*, **28**, 957–964.

Role of α-adrenoceptors in the spinal cord in myocardial infarction-induced cardiac arrhythmias

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The central nervous system has been implicated in the genesis of cardiac arrhythmias following coronary arterial ligation (CAL), since cardiac denervation prevented their development (Schael, Wallace & Seally, 1969) and increased sympathetic nerve activity was demonstrated after CAL (Brown & Malliani, 1971). Bhargava, Srivastava, Barthwal & Sinha (1976) showed the importance of the thoracic spinal cord for production of CAL-evoked cardiac arrhythmias. Histofluorescene studies revealed the existence of bulbospinal catecholamine pathways to intermedio-lateral cell column (Dahlstrom & Fuxe, 1965). Therefore, the present study was undertaken to elucidate the role of α-adrenoceptors in the spinal cord which may modulate the genesis of CAL-evoked cardiac arrhythmias.

Experimental myocardial infarction was produced in dogs by ligation of the left anterior descending coronary artery (Harris & Kokernot, 1950). Ventricular ectopic beats appeared after 24 h of CAL. At this stage, spinal cord transection (C_1) was done under chloralose anaesthesia. Adrenoceptor agonists and

Table 1 Effect of α-adrenoceptor agonists and antagonists on CAL evoked cardiac arrhythmias in dogs with spinal cord transected at C_1

Drug		*Dose/route*	*Pretreatment*	*Mean % arrhythmia ± s.e. mean* *Before drug treatment*	*After drug treatment*
Normal saline	(3)	0.5 ml i.t.	—	61.9 ± 10.1	69.0 ± 11.1
Clonidine	(4)	10 μg i.t.	—	50.8 ± 8.7	16.2 ± 4.2*
	(4)	10 μg i.v.	—	31.5 ± 11.2	24.9 ± 5.2
	(1)	40 μg i.v.	—	34.0	100.0
Phenylephrine	(3)	200 μg i.t.	—	69.9 ± 2.1	26.0 ± 11.3*
	(5)	200 μg i.v.	—	50.5 ± 12.3	87.1 ± 7.8*
Piperoxan	(6)	500 μg i.t.	—	44.3 ± 4.5	86.8 ± 4.8*
Clonidine	(3)	10 μg i.t.	Piperoxan 500 μg i.t.	80.0 ± 4.5	80.7 ± 5.9

* $P < 0.05$.
No. in parentheses indicate No. of animals.

antagonists were administered either intrathecally (i.t.) or intravenously (i.v.). The percent arrhythmia before and after drug pretreatment was analysed according to the method of Bhargava *et al.* (1976) and the level of significance determined by Student's '*t*' test.

Results of the present study are shown in Table 1.

Intrathecal injection of α-adrenoceptor agonists, clonidine (10 μg) and phenylephrine (200 μg) antagonised the CAL-evoked cardiac arrhythmias ($P < 0.05$). On the other hand, i.v. injection of clonidine (10 μg) did not significantly effect CAL-evoked arrhythmias. Clonidine (40 μg i.v.) and phenylephrine (200 μg i.v.) were found to potentiate CAL-induced arrhythmia. A similar potentiation ($P < 0.05$) of CAL-evoked arrhythmia was observed with the α-adrenoceptor antagonist piperoxan (500 μg i.t.). However, intrathecal piperoxan pretreatment prevented the blockade of cardiac arrhythmia by intrathecal clonidine. Thus it is concluded that α-adrenoceptors present in the spinal cord are inhibitory to CAL-evoked cardiac arrhythmias. A potentiation of CAL-evoked arrhythmia by a peripheral α-adrenoceptor agonist may be due to stimulation of peripheral α-adrenoceptors resulting in alteration in coronary circulation or to an increase in arterial pressure. Pant, Gurtu, Tangri & Bhargava (1980) have earlier shown that β-adrenoceptors in the spinal cord are excitatory for CAL-evoked cardiac arrhythmias.

References

BHARGAVA, K.P., SRIVASTAVA, V.K., BARTHWAL, J.P. & SINHA, J.N. (1976). Significance of spinal autonomic loci in post-coronary ligation arrhythmia. *Neuropharmacology*, **15**, 625–633.

BROWN, A.M. & MALLIANI, A. (1971). Spinal sympathatic reflexes initiated by coronary receptors. *J. Physiol.*, **212**, 685–705.

DAHLSTROM, A. & FUXE, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system—II. Experimentally induced changes in intraneuronal amine levels of bulbospinal neurone systems. *Acta. Physiol. Scand.*, **64**, Suppl. 247, 5–36.

HARRIS, A.S. & KOKERNOT, R.H. (1950). Effects of diphenylhydantoin sodium (dilantin sodium) and phenobarbital sodium upon ectopic ventricular tachycardia in acute myocardial infarction. *Am. J. Physiol.*, **163**, 505–516.

PANT, K.K., GURTU, S., TANGRI, K.K. & BHARGAVA, K.P. (1980). Role of spinal β-adrenoceptors in cardiac arrhythmias. *Indian J. Pharmacol.*, **11**, 4. In press.

SCHAAL, S.F., WALLACE, A.G. & SEALY, W.C. (1969). Protective influence of cardiac denervation against arrhythmias of myocardial infarction. *Cardiovas. Res.*, **3**, 241–244.