PROCEEDINGS OF THE

BRITISH PHARMACOLOGICAL SOCIETY

4TH–5TH JANUARY, 1968

COMMUNICATIONS

In communications with more than one author, an asterisk (*) denotes the one who presented the work.

Evidence for a direct action of antidiuretic hormone

By A. W. CUTHBERT and ELISABETH PAINTER*, *Department of Pharmacology, University of Cambridge*

There is a considerable amount of evidence to suggest that antidiuretic hormone acts indirectly to stimulate active sodium transport (see Orloff & Handler, 1967). Most of the evidence has been obtained from sodium transporting epithelia such as the frog skin and toad bladder.

Basically the theory proposes that antidiuretic hormone increases the activity of adenyl cylase, thus increasing the rate of formation of 3′,5′-AMP from ATP. The cyclic nucleotide is then thought to alter the permeability of the apical membrane to sodium ions which enter the cell to be subsequently removed by the sodium pump situated in the basal membrane.

Evidence for the theory is based on the following facts: (1) the effects of the cyclic nucleotide and antidiuretic hormone on active sodium transport in transporting epithelia are indistinguishable; (2) methylxanthines, such as theophylline, also mimic the effect of antidiuretic hormone by inhibiting an intracellular cyclic nucleotide phosphodiesterase, which degrades 3′,5′-AMP to inactive 5′-AMP; (3) metabolic inhibitors inhibit the actions of antidiuretic hormone.

An alternative view of antidiuretic hormone action is that it acts directly on the apical membrane to increase sodium permeability, and that the increased sodium influx results in the formation of 3′-5′-AMP, activation of phosphorylase and formation of high energy phosphates for the pump mechanism.

A crucial test for either hypothesis seemed to be one which would measure the effect of antidiuretic hormone, 3′,5′-AMP and theophylline on the permeability of the apical membrane directly, without depending on active sodium transport across the epithelium. For this purpose the "current clamped" frog skin preparation has been developed. The technique consists of applying current to the skin to maintain the normal ionic gradients while active transport of sodium is inhibited by ouabain or metabolic inhibitors. Eventually both the applied current and the skin potential become constant. From theory

it is shown that, when the skin is "current clamped" at the steady state, an increase in permeability of either the apical or basal cell membrane will cause a rise in skin potential. Antidiuretic hormone was found to cause a potential increase in the absence of active sodium transport in accord with the theoretical expectation. The same result was obtained with skins treated with sodium azide and deprived of oxygen. Cyclic 3',5'-AMP caused only a minor transitory potential increase, whereas theophylline caused a large decrease in skin potential.

Because the hormone, cyclic nucleotide and theophylline do not produce similar responses, as they do when active sodium transport is the index of action, the metabolic mechanism of action of antidiuretic hormone must be questioned.

REFERENCE

ORLOFF, J. & HANDLER, J. (1967). The role of adenosine 3', 5'-phosphate in the action of antidiuretic hormone. *Am. J. Med.*, **42**, 757–768.

The continuous estimation of vasopressin in the circulating blood

By N. J. GILMORE* and J. R. VANE, *Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2*

The isolated rectum from the rabbit is a sensitive and reasonably specific assay organ for the estimation of vasopressin by the blood-bathed organ technique (Vane, 1964). Vasopressin (Pitressin, 25 μu.–500 μu./ml.), oxytocin (Pitocin, 750 μu.–1·5 u./ml.) and the catecholamines (2–100 ng/ml.) were the only substances found to relax the tissue. Many other substances caused contraction, including 5-hydroxytryptamine, angiotensin II, acetylcholine, bradykinin, prostaglandins E_1, E_2, $F_{2\alpha}$ and substance P, whereas histamine, SRS-A and pentagastrin had no effect.

Vasopressin was approximately 30 times more potent than oxytocin. Isoprenaline was the most potent of the catecholamines, followed by noradrenaline and adrenaline respectively. The inhibitory effects of these substances could be antagonized by propranolol (10^{-7} g/ml.) without the action of vasopressin being affected.

The half-life of vasopressin in dog's blood at 37° C was measured in a circuit of silicone tubing interposed between the dog and the isolated assay organ. This showed that 50% of the activity of vasopressin disappeared in 3·5 min. There was no disappearance in similar incubation experiments with Krebs solution.

Vasopressin survived passage through the pulmonary circulation without detectable loss but 40% of the inhibitory activity on the blood-bathed isolated rectum disappeared in one circulation through the rest of the body. Assuming that a complete circulation takes less than 20 sec (Spector, 1956), this result indicates that the half-life of vasopressin in the circulation of the dog is approximately 30 sec. Other workers (Silver, Schwartz, Fong, Debons & Dahl, 1961; Lauson & Bocanegra, 1961; Share, 1962) using either radioactivity or antidiuretic activity as an assay, have found a half-life of vasopressin in the circulation of the dog of about 5 min. The difference between this figure and our value of 30 sec may

be the result of such factors as the duration of infusion, protein binding in the plasma, or metabolism.

Supported by a grant from Smith & Nephew Limited.

REFERENCES

LAUSON, H. D. & BOCANEGRA, M. (1961). Clearance of exogenous vasopressin from plasma of dogs. *Am. J. Physiol.*, **200**, 493–497.

SHARE, L. (1962). Rate of disappearance of arginine vasopressin from circulating blood in the dog. *Am. J. Physiol.*, **203**, 1179–1181.

SILVER, L., SCHWARTZ, I. L., FONG, C. T. O., DEBONS, A. F. & DAHL, L. K. (1961). Disappearance of plasma radioactivity after injection of H^3- or I^{131}-labelled arginine vasopressin. *J. appl. Physiol.*, **16**, 1097–1099.

SPECTOR, W. S. (ed.) (1956). *Handbook of Biological Data*, pp. 285. Philadelphia: Saunders.

VANE, J. R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmac. Chemother.*, **23**, 360–373.

The effect of various steroids on pituitary-adrenal function in the rat

By J. D. FLACK and M. A. STOCKHAM*, *Department of Pharmacology, School of Pharmacy, Brunswick Square, London, W.C.*1, *and Sherrington School of Physiology, St. Thomas's Hospital Medical School, London, S.E.*1

A study of synthetic and naturally occurring steroids which have oestrogenic, androgenic, progestogenic and anabolic activity on pituitary–adrenal function has been undertaken in the rat. An attempt was made to produce some changes in pituitary–adrenal function seen in male and female rats in various physiological conditions by administering these groups of steroids and testing the responses of the rats in quiescent and stress conditions. The parameters of pituitary–adrenal function used were plasma and adrenal contents of corticosterone, *in vitro* corticosteroid genesis, and plasma protein binding activity for corticosterone in the blood. The results suggested that androgen, oestrogen or progestogen dominance could alter corticosterone metabolism by altering activities of the pituitary and adrenal glands and the peripheral distribution of the steroids and thus mimic some physiological variations. The doses of steroids were critical, however, because a dose-dependent stimulation or inhibition of pituitary–adrenal axis could be obtained.

Some effects of chronic alcohol administration in the rat

By J. CROSSLAND and F. RATCLIFFE*, *Pharmacology Laboratories, University of Nottingham*

Rats whose drinking water contained progressively increasing quantities of ethanol (2·5 to 25% by volume over 7 weeks) increased in body weight at a much slower rate than untreated litter mates. The body weight increased rapidly when the ethanol was withdrawn. Measurements of body composition and determination of the mutual correlations between food intake, fluid intake, body weight, and skeletal length all suggested that normal growth is inhibited during ethanol administration and that following withdrawal of the drug the inhibition is removed.

The inhibition of growth in rats treated with ethanol might have been caused by a depression of hypothalamic function; this possibility was investigated in untreated and alcohol-treated rats.

It is known that a single intraperitoneal dose of ethanol has a stressing effect in rats which results in an increased concentration of plasma corticosterone; this effect is caused by a direct action on the hypothalamus. The corticosterone response in animals so treated parallels the curve of blood alcohol concentration. This technique was therefore used as a means of assessing hypothalamic sensitivity in untreated and ethanol-treated rats.

The effect of a single dose of ethanol on plasma corticosterone levels was significantly greater in untreated animals than in those which had received alcohol in their drinking water for several weeks. Moreover the fall in adrenal ascorbic acid concentration was significantly greater in the untreated animals.

These findings suggest that the hypothalamus of alcohol-treated animals is either depressed or has developed a tolerance to ethanol. Evidence in support of the former explanation was that resting plasma corticosterone levels were lower and adrenal ascorbic acid levels higher in alcohol-treated animals than in untreated animals.

The response of untreated and alcohol-treated rats to auditory stimulation and to hypothermia induced by chlorpromazine was also studied. Plasma corticosterone and adrenal ascorbic acid levels were determined 1 hr after auditory stimulation. The plasma corticosterone reached a significantly higher concentration in the untreated animals. The untreated animals also suffered a greater loss of ascorbic acid from their adrenal glands.

The hypothermic response of alcohol-treated rats to the intraperitoneal injection of chlorpromazine was significantly less than that of untreated animals. Moreover, the basal body temperature of the animals given alcohol was significantly lower than that of untreated litter mates.

These results suggest that the retardation of growth of rats produced by the chronic administration of ethanol is a manifestation of a generalized depression of hypothalamic function.

This work was supported by a grant from the Medical Research Council.

Depression of frog motoneurones by volatile anaesthetic agents

By A. RICHENS (introduced by J. P. QUILLIAM). *Department of Pharmacology, St. Bartholomew's Hospital Medical School, London, E.C.1.*

The action of several volatile anaesthetic agents (chloroform, 0·5 mg/ml., halothane 0·5 mg/ml. and ethyl chloride, 1·5 mg/ml.) has been observed on the discharge of motoneurones in the isolated spinal cord of the frog. The discharge was produced by two distinct excitatory pathways; monosynaptically by lateral funiculus stimulation and polysynaptically by dorsal root stimulation. Recordings from a ventral root with suction electrodes showed that both types of discharge were equally susceptible to depression by these agents. The failure of discharge seemed to result from an increase in the threshold of the motoneurones to monosynaptic excitation, accompanied by a reduction in the rate

of rise of the synaptic potential. These observations have been confirmed by intracellular recording from motoneurones.

Focal recording from dorsal and ventral horns with a coarse extracellular microelectrode revealed no impairment of conduction in primary afferent or lateral funiculus fibres in normal anaesthetic concentrations, but doubling or trebling the concentration produced a marked conduction block. The ability of antidromic impulses in the ventral root to invade the motoneurone somas was impaired by normal concentrations, and the excitability of the motoneurones to direct stimulation through the microelectrode was depressed. These observations would be satisfactorily explained by suggesting that the depression of reflex activity results from a direct action on the motoneurone membrane. A stabilization of the initial segment would account for the rise in threshold to orthodromic, antidromic and direct stimulation, whereas the depression of the synaptic potential must result from a stabilization of the subsynaptic membrane. A reduction in the output of the excitatory transmitter cannot, however, be excluded.

Some biochemical and pharmacological properties of phencyclidine

By B. E. LEONARD and SALLY TONGE*, *Department of Pharmacy, University of Nottingham*

The synthetic cyclohexylamine, phencyclidine, was introduced into anaesthetic practice because of its ability to produce analgesia without loss of consciousness (Greifenstein, De Vault, Yoshitake & Gatewski, 1958). Post-operatively, however, symptoms that resembled some aspects of schizophrenia commonly occurred (Meyer, Greifenstein & De Vault, 1959) and as a consequence the clinical use of this drug has largely been discontinued. Nevertheless, as phencyclidine is structurally unrelated to any of the other major hallucinogens it was of interest to investigate some of its biochemical properties in detail. The general pharmacological properties of this drug were first reported by Chen, Ensor, Russell & Bohner (1959) and reviewed by Domino (1964).

Following intraperitoneal injection into rats or mice, phencyclidine (10 mg/kg) causes excitement, stereotyped behaviour and, in rats, causes the animals repeatedly to walk backwards. The marked ataxia seen in rats after parenteral injection is possibly a consequence of a slight neuromuscular blocking action of the drug. These behavioural effects also occur after intraventricular injection of the drug but the ataxia is less pronounced. After intraperitoneal injection these symptoms persist for approximately 5 hr. Our studies so far have been on the changes in possible neurotransmitter substances in the rat brain in an attempt to determine which of these substances are correlated with the behavioural changes that occur during this period.

Brain 5-hydroxytryptamine concentration rose by 40% 5 min after injection, fell to approximately normal levels and then slowly rose again to 37% above control after 2 hr. 5-Hydroxyindole acetic acid concentration fell by 23% shortly after injection but 30 min later rose so that the changes mirror those of 5-hydroxytryptamine. A fall in noradrenaline concentration (23% after 30 min) was accompanied by a rise in that of dopamine (8% after 30 min); the concentrations returning to normal over 5 hr. Concentrations of γ-amino butyric acid, and to a lesser extent glutamic and aspartic acids, decreased throughout the period of drug action. Brain concentrations of histamine, acetylcholine and high

energy phosphate compounds were unaffected as a consequence of the action of phencyclidine. There was an increase in the activity of 5-hydroxytryptophan decarboxylase, but no change in cholinesterase activity.

REFERENCES

CHEN, G., ENSOR, C. R., RUSSELL, D. & BOHNER, B. M. (1959). The pharmacology of 1-(1-phenylcyclohexyl) piperidine HCl. *J. Pharmac. exp. Ther.*, **127**, 241–250.

DOMINO, E. F. (1964). Neurobiology of phencyclidine (sernyl), a drug with an unusual spectrum of pharmacological activity. *Int. Rev. Neurobiol.*, **6**, 303–347.

GREIFENSTEIN, F. E., DE VAULT, M., YOSHITAKE, J. & GATEWSKI, J. E. (1958). Study of 1-aryl-cyclohexylamine for anaesthesia. *Anaesth. Analg. Curr. Res.*, **37**, 283–294.

MEYER, J. S., GREIFENSTEIN, F. & DE VAULT, M. (1959). A new drug causing symptoms of sensory deprivation. *J. nerv. ment. Dis.*, **129**, 54–61.

The effect of stimulant drugs on the release of acetylcholine from the cerebral cortex

By B. A. HEMSWORTH and M. J. NEAL*, *Department of Pharmacology, University of Cambridge*

Experiments on anaesthetized animals have shown that, in the presence of an anticholinesterase, acetylcholine is released from the cerebral cortex. There is strong evidence that the acetylcholine is released from cholinergic nerve endings in the brain and is therefore a result of neuronal activity. Mitchell (1963) found that the release of acetylcholine from the cat cerebral cortex was reduced during deep anaesthesia and increased by the parenteral administration of leptazol.

In the present experiments, a range of centrally acting stimulant drugs which increase neuronal activity at different sites of the neuraxis was used to investigate further the relationship between increased neuronal activity and release of acetylcholine from the cerebral cortex.

The cerebral cortex of anaesthetized rats was widely exposed bilaterally. The head was clamped and a Perspex cylinder (vol. 1 ml.) was placed on each cerebral hemisphere immediately after removal of the underlying dura mater. Ringer–Locke solution containing eserine (100 μg/ml.) and atropine (0·4 μg/ml.) was placed in the cylinders. The solution was removed after 30 min and assayed for acetylcholine using the dorsal muscle of the leech.

Picrotoxin (12 mg/kg I.P.) and leptazol (300 mg/kg I.P.) produced large increases in acetylcholine release, the maximum release after these drugs being about seven times the resting release. Nikethamide, which is a weak convulsant drug, in large doses (2 g/kg I.P.), increased the acetylcholine release to twice the resting level.

Strychnine, even in high doses (12 mg/kg I.P.), produced only a small increase in acetylcholine release to approximately twice the resting release. Dexamphetamine (100 mg/kg I.P.) increased the release of acetylcholine to about three times the resting release, whereas caffeine (100 mg/kg I.P.) produced no significant change.

The results suggest that the drugs with powerful sub-cortical actions such as leptazol and picrotoxin are more effective in stimulating the release of acetylcholine than are dexamphetamine and caffeine which are thought to act primarily on the reticular formation and cerebral cortex. The results support previous suggestions that stimulation of subcortical structures is a major factor in increasing output from cortical cholinergic fibres.

The comparatively small effect of strychnine is probably because the main site of action of this drug is the spinal cord.

REFERENCE

MITCHELL, J. F. (1963). The spontaneous and evoked release of ACh from the cerebral cortex. *J. Physiol., Lond.*, **165**, 98–116.

Characteristics of the response of brain-stem neurones to noradrenaline

By R. J. BOAKES, P. B. BRADLEY, N. BROOKES* and J. H. WOLSTENCROFT, *Medical Research Council, Neuropharmacology Research Unit and Department of Physiology, The Medical School, University of Birmingham*

The technique of iontophoretic application of drugs from multibarrelled micropipettes has been used to study responses of brain-stem neurones in decerebrate cats. The effects of (−)-noradrenaline on the firing rate of spontaneously active neurones have been found to conform to certain well defined patterns.

Excitation is almost invariably long-lasting and delayed in onset. This response shows varying degrees of "desensitization". Inhibition is usually of comparatively rapid onset, and recovery is complete soon after the applying current is switched off. In some cases excitation is preceded by this type of inhibition. Another form of inhibition, less frequently observed, is similar to the excitatory response in that it lasts much longer than the period of application and reaches its peak after the current has been switched off. In contrast to excitation, inhibitory responses are rarely seen to diminish with repeated application of (−)-noradrenaline.

It is possible that some of the effects observed when (−)-noradrenaline is applied iontophoretically are indirect, for example, via an action on a neighbouring neurone too small to be recorded. In an attempt to localize the effects of (−)-noradrenaline and to record from smaller neurones, some experiments were made in which weak currents were passed through electrodes with small tips (overall diameter 3–5 μ). In these experiments the effects obtained were similar to those observed with the larger electrodes. These results, together with findings from experiments in which agonists and antagonists were used, provide evidence against indirect effects from neighbouring neurones.

(+)-Noradrenaline inhibited neurones which were inhibited by (−)-noradrenaline, but on neurones excited by (−)-noradrenaline its effect was either weaker or absent. Thus the excitatory effect shows stereospecificity whereas the inhibitory effect does not.

All types of response to (−)-noradrenaline have proved resistant to block by dichloroisoprenaline or propranolol. (−)-Isoprenaline was almost devoid of effect on the neurones under study. Dihydroergotamine (but not phentolamine) occasionally antagonized excitatory responses to (−)-noradrenaline but this action was less consistent than that of chlorpromazine (Bradley, Wolstencroft, Hösli & Avanzino, 1966). These results suggest that the receptors for noradrenaline on brain-stem neurones are of more than one kind and that they do not fit into the α and β classification applied to peripheral receptors.

REFERENCE

BRADLEY, P. B., WOLSTENCROFT, J. H., HÖSLI, L. & AVANZINO, G. L. (1966). Neuronal basis for the central action of chlorpromazine. *Nature, Lond.*, **212**, 1425–1427.

On the mechanism of the hyperthermia produced by reserpine injected into the cerebral ventricles of rabbits

By U. BANERJEE†, T. F. BURKS*, W. FELDBERG and CECILIE A. GOODRICH‡, *The National Institute for Medical Research, Mill Hill, London, N.W.7*

Recently, Cooper, Cranston & Honour (1967) found that an injection of 0·35–0·75 mg reserpine into the cannulated lateral ventricle of the unanaesthetized rabbit produced a rise in temperature and led to depletion of the monoamines in the hypothalamus, as evidenced by the disappearance of the fluorescence characteristic for noradrenaline. They discussed the possibility of the rise resulting from release of noradrenaline by reserpine. One way of testing this possibility would be to see if the temperature raising effect of reserpine is lost after depletion of the monoamines as a result of its first injection.

It was found that in an unanaesthetized rabbit in which the first injection of 0·5–0·6 mg reserpine phosphate (kindly supplied by Dr. A. J. Plummer, Ciba Pharmaceutical Company, Summit, N.J., U.S.A.) into the lateral ventricle through a chronically implanted cannula caused a rise in temperature; a second similar injection given 24 hr later was without any effect on temperature. This condition persisted for at least 5 days.

This finding supports the view expressed by Cooper *et al.* (1967) that the rise in temperature produced by a first intraventricular injection of reserpine results from release of noradrenaline.

† Commonwealth Medical Fellow.

* Supported by U.S.A. Public Health Service Fellowship, No. 1-F2-GM-22,826-02, from the National Institute of General Medical Sciences.

‡ Supported by U.S.A. Public Health Service Fellowship, No. 1-F2-NB-32,251-01 NSRB, from the National Institute of Neurological Diseases and Blindness.

REFERENCE

COOPER, K. E., CRANSTON, W. I. & HONOUR, A. J. (1967). Observations on the site and mode of action of pyrogens in the rabbit brain. *J. Physiol., Lond.*, **191**, 325–337.

The reversal of the central effects of noradrenaline by antidepressant drugs in mice

By PAULINE COWELL* and M. J. DAVEY, *Therapeutics Research Division, Pfizer Limited, Sandwich, Kent*

Brittain (1966) reported that pretreatment with antidepressant drugs antagonized the depressive and hypothermic actions of noradrenaline introduced into the lateral cerebral ventricles of mice. He concluded that it was impossible to reconcile this antagonism of noradrenaline by antidepressant compounds with the current concept that these agents facilitate central adrenergic mechanisms.

An attempt was therefore made to correlate this antagonistic action of imipramine-like compounds with alterations in either the uptake, metabolism or subcellular distribution of centrally administered ^{3}H-noradrenaline. Pretreatment with nortriptyline prevented the hypothermia but failed to modify either the uptake or the subcellular distribution of ^{3}H-noradrenaline. There was, however, a slight increase in O-methylated metabolites of noradrenaline in the pretreated mice. The finding that the antagonistic potency of nortriptyline administered directly into the lateral ventricle was of the same order as that

obtained following peripheral administration further mitigated against a central action for nortriptyline in this situation.

It was decided to investigate the possibility that this action of imipramine-like antidepressant compounds could be a consequence of their peripheral effects rather than a direct central antagonism of noradrenaline.

Noradrenaline (0·25–2·0 mg/kg) given subcutaneously caused hyperthermia in mice. This hyperthermia was found to be markedly potentiated by imipramine-like antidepressant compounds, in agreement with the results of Jori, Paglialunga & Garattini (1967).

Noradrenaline (10 μg) given into the lateral ventricles of mice induced hypothermia. This hypothermia was reversed by noradrenaline (0·25–2·0 mg/kg) given subcutaneously, as it was by imipramine-like antidepressant drugs. In addition, the reversal of the hypothermia was antagonized by α and β receptor blocking agents whether the reversal was induced by subcutaneous noradrenaline or by imipramine-like drugs.

One explanation of these findings is that part of the noradrenaline given into the lateral cerebral ventricle passes to the periphery via the choroid plexus, and that it is the potentiation of the peripheral effects of this portion by imipramine-like compounds that is responsible for the reversal of the noradrenaline hypothermia. Noradrenaline (10 μg) given centrally caused a rapid and prolonged pressor response in mice anaesthetized with sodium pentobarbitone. This pressor response was not antagonized, but in fact was potentiated by ganglionic blocking doses of hexamethonium and pempidine, and was therefore not the result of centrally evoked increased sympathetic activity.

Nortriptyline (0·125–1·0 mg/kg) given either centrally or peripherally markedly potentiated the pressor responses to both centrally and peripherally administered noradrenaline in anaesthetized mice, at the same dose levels required to antagonize the centrally induced hypothermic action of noradrenaline.

It is therefore suggested that the antagonism of the hypothermia caused by centrally administered noradrenaline by imipramine-like antidepressant compounds results from their well documented peripheral blockade of the uptake of noradrenaline.

REFERENCES

BRITTAIN, R. T. (1966). The intracerebral effects of noradrenaline and its modification by drugs in the mouse. *J. Pharm. Pharmac.*, **18**, 621–623.

JORI, A., PAGLIALUNGA, S. & GARATTINI, S. (1967). Effect of antidepressant and adrenergic blocking drugs on hypothermia induced by reserpine. *Archs int. Pharmacodyn. Thér.*, **165**, 384–393.

The effects of chlorpromazine and reserpine in hyperthyroid mice

By P. S. J. SPENCER and R. WAITE*, *Department of Pharmacy, The University of Aston, Birmingham*

It is known that changes in the hormonal balance of animals can affect their behaviour, and also their responsiveness to the peripheral actions of both endogenous and exogenous substances. Yet very little is known about the actions of centrally acting drugs in animals with hormonal imbalance.

The pharmacological effects of chlorpromazine and reserpine have been studied in mice made hyperthyroid with thyroxine. Using a strain of TO albino male mice, a maximal degree of hyperthyroidism was produced with injections of thyroxine (2 mg/kg/day) for 10 days. On the eleventh day, the effects of chlorpromazine and reserpine on body temperature and spontaneous locomotor activity were determined. At an ambient temperature of 22° C, the hypothermia caused by chlorpromazine (5 mg/kg I.P.) was substantially less in the hyperthyroid mice than in euthyroid controls. Similarly hypothermia induced by reserpine (0·5 mg/kg I.P.) was less in the hyperthyroid animals. In contrast, both drugs markedly reduced spontaneous locomotor activity, irrespective of the thyroid state of the animal.

The severe hypothermia which chlorpromazine and reserpine cause in normal mice is thought to be the result of two simultaneous effects of drug action: a fall in heat production, caused by the locomotor inactivity and the consequent loss of muscular heat; and also an inhibition of hypothalamic heat control mechanisms, so that no compensation is made for the fall in heat production. If this is so, simply increasing the rate of heat production in immobile animals, for example, by raising the basal metabolic rate, should reduce or prevent the hypothermic actions of these drugs. We believe that this satisfactorily explains the actions of thyroxine treatment in these mice. It also follows that if the rate of heat loss of immobile animals is reduced, for example by raising the laboratory temperature, this also should reduce the degree of hypothermia. This was observed in control animals given either chlorpromazine or reserpine.

Finally, we examined the effects of these two drugs in hyperthyroid mice at a raised ambient temperature (26° C). Both chlorpromazine and reserpine produced hyperthermia in the hyperthyroid mice, yet continued to reduce markedly spontaneous locomotor activity.

We conclude that both chlorpromazine and reserpine abolish hypothalamic control of body temperature; whether or not this is followed by hypothermia or hyperthermia depends on the balance between heat loss and heat gain. Although a severe hypothermic state enhances it, the depression of spontaneous locomotor activity is not dependent on the presence of hypothermia.

Bradykinin and the Philipeaux–Vulpian phenomenon

By B. RADMANOVIC and SONIA H. TORRES* (introduced by J. H. WOLSTENCROFT), *Department of Physiology, The Medical School, University of Birmingham*

When the hypoglossal nerve is sectioned and allowed to degenerate, the musculature of the tongue becomes responsive to chordo-lingual nerve stimulation, giving rise to a contracture (the Philipeaux–Vulpian phenomenon). Dale & Gaddum (1930) concluded that acetylcholine is responsible for this effect, but added that it could also be attributed to "any substance sharing the dual action of acetylcholine, causing dilation of normal arterioles and contracture of denervated voluntary muscles." More recently, Hilton & Lewis (1958) found, in the normal tongue, that chordo-lingual nerve stimulation released kinin-forming enzyme from the glandular elements of the tongue. Thus, if active, bradykinin might contribute to the contractile response of the denervated tongue on chorda stimulation.

Cats were anaesthetized with chloralose, 6–9 days after hypoglossal nerve section. The contractile response of the tongue was registered with a strain-gauge. The tongue

always responded to close arterial injections of bradykinin, most frequently by contraction but sometimes by a contracture or a two-phase response. The threshold dose was between 50 and 400 ng. The strongest contraction was usually obtained with 200–250 ng, the response diminishing if the dose was increased. Responses of similar magnitude could be elicited by 2 ng acetylcholine and by chordo-lingual stimulation with pulses at 30/sec (1 msec duration and 15 V). The response to acetylcholine increased with increasing doses.

Close arterial injections of (+)-tubocurarine and gallamine, in doses sufficient to reduce to one-third to one-quarter the contraction produced by stimulation of the contralateral hypoglossal nerve (0·25–1 mg and 0·25–4 mg, respectively), produced a large contracture that lasted 1–15 min. After the tongue relaxed the effects of bradykinin, acetylcholine and chordo-lingual stimulation were all reduced: bradykinin 30–75%; acetylcholine 60–90%; and chorda stimulation 35–100%.

These results show that bradykinin could be contributing to the Philipeaux–Vulpian phenomenon. Its action on denervated striated muscle may be direct; in the denervated tongue it could also act by releasing acetylcholine from parasympathetic nerve endings. These possibilities were tested in two ways. First, bradykinin was injected close arterially into a denervated skeletal muscle: in two out of seven experiments it produced contraction of the denervated tibialis anterior; in the other five it potentiated the response to subsequent injections of acetylcholine. Secondly, other vasodilator substances were tested on the denervated tongue: close arterial injections of either sodium nitrite (1 mg) or isoprenaline (40–200 ng) produced contractile responses which were abolished by (+)-tubocurarine.

After denervation, therefore, the tongue muscles like other striated muscles (Alonso De Florida, Del Castillo, Gonzalez & Sanchez, 1965) probably react directly to many substances including bradykinin.

REFERENCES

ALONSO DE FLORIDA, F., DEL CASTILLO, J., GONZALEZ, CARMEN C. & SANCHEZ, V. (1965). Anaphylactic reaction of denervated skeletal muscle in the guinea-pig. *Science*, **147**, 1155–1156.

DALE, H. H. & GADDUM, J. H. (1930). Reactions of denervated voluntary muscle and their bearing on the mode of action of parasympathetic and related nerves. *J. Physiol., Lond.*, **70**, 109–144.

HILTON, S. M. & LEWIS, G. P. (1958). Vasodilation in the tongue and its relationship to plasma kinin formation. *J. Physiol., Lond.*, **144**, 532–540.

Afferent impulses in the hepatic nerve of perfused livers elicited by 5-hydroxytryptamine and other substances

By W. H. H. ANDREWS, *Department of Experimental Surgery, London Hospital Medical College, London, E.*1

Livers from rabbits were perfused via the portal vein with Krebs solution at 40–50 ml./min at approximately 36° C. There was no recirculation. Injections of drugs were made close to the portal cannula. Slips of the hepatic nerve were dissected off the wall of the portal vein and divided. The distal end was laid across wick electrodes, and impulses arising in the liver were recorded.

Spontaneous afferent impulses frequently occurred throughout the course of experiments, which lasted up to 6 hr. Acetylcholine (>5 μg) evoked a nervous discharge: im-

pulses began within 1 sec of injection and lasted a few seconds. A second injection often produced a greater effect. With 5-hydroxytryptamine (>0·2 μg) there was a similar response, except that slight tachyphylaxis was observed. With bradykinin, more than 2·0 μg was needed to produce a nervous discharge: there was a delay of a few seconds before firing began, and the response lasted for 2–5 min. Tachyphylaxis was pronounced. Occasionally histamine (up to 100 μg) evoked a nervous discharge: there was a delay of about 18 sec before firing began, and the response lasted for several minutes. Tachyphylaxis was present. None of the responses appeared to be secondary to vascular changes, and it was shown that 5-hydroxytryptamine and bradykinin activated different fibres. It was anticipated, on the basis of experiments carried out by Guzman, Braun & Lim (1962), who recorded vocalization of lightly anaesthetized dogs, that bradykinin would be more active than 5-hydroxytryptamine.

Professor Eric Neil kindly provided facilities for the experiments to be carried out in his department.

REFERENCE

GUZMAN, F., BRAUN, C. & LIM, R. K. S. (1962). Visceral pain and the pseudo-afferent response to intra-arterial injection of bradykinin and other algesic agents. *Archs int. Pharmacodyn. Thér.*, **134**, 353–384.

The actions of choline, adrenaline and phenoxybenzamine on the innervated longitudinal muscle strip of the guinea-pig ileum

By H. W. KOSTERLITZ and R. J. LYDON*, *Department of Physiology, University of Aberdeen, Scotland*

The innervated longitudinal muscle strip (Rang, 1964) was bathed in Krebs solution containing hexamethonium bromide (70 μM) and mepyramine maleate (0·125 μM). After supramaximal stimulation (0·5 msec pulse duration, 6 pulses/min) for 2–3 hr, the height of the contractions decreases and the depressant effect of adrenaline increases. When, at this point, choline chloride (10–20 μM) is added to the bath, the height of the twitch and the sensitivity to adrenaline return to normal. This amount of choline increases the sensitivity of the strip to acetylcholine, the dose ratio being about 0·7. In the absence of choline, the output of acetylcholine diminishes progressively, at rest and during stimulation; addition of choline restores the output to its original level. Adrenaline depresses acetylcholine output at rest and during stimulation; addition of choline reduces this effect of adrenaline. Exposure of the strip to phenoxybenzamine (3 μM for 20 min) antagonizes the depressant action of adrenaline and noradrenaline on acetylcholine output, a finding which confirms the observations of Watt (1966) on the whole ileum and of Vizi (1967) on the strip. Because phenoxybenzamine blocks the acetylcholine receptors in the muscle, it is impossible to study the antagonistic action of phenoxybenzamine on the depressant effects of adrenaline on the responses of the preparation to electrical stimulation. Protection of the acetylcholine receptors by high concentrations of acetylcholine (0·15 mM) during exposure to phenoxybenzamine makes it possible to demonstrate this antagonism of adrenaline by phenoxybenzamine.

Supported by U.S. Public Health Service grant NB 03026.

REFERENCES

RANG, H. P. (1964). Stimulant actions of volatile anaesthetics on smooth muscle. *Br. J. Pharmac. Chemother.*, **22**, 356–365.

VIZI, E. S. (1967). The inhibitory action of noradrenaline and adrenaline on release of acetylcholine from guinea-pig ileum longitudinal strips. Proceedings of meeting of British and German Pharmacological Societies, Cambridge. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, in the Press.

WATT, A. J. (1966). Inhibitory mechanisms in guinea-pig isolated ileum. Ph.D. Thesis, Univ. Aberdeen.

Bretylium on C fibre excitation and noradrenaline release by acetylcholine and electrical stimulation

By M. J. DAVEY*, M. L. HAYDEN and P. C. SCHOLFIELD, *Therapeutics Research Division, Pfizer Limited, Sandwich, Kent*

The effects of bretylium on the excitation of postganglionic adrenergic C fibres by acetylcholine and on the release of the sympathetic transmitter by acetylcholine and by electrical stimulation was studied using the *in situ* preparation of the cat spleen described by Brown & Gillespie (1957), and the cross-perfused isolated cat spleen described by Abercrombie, Davies & Dwyer (1962).

As was first observed by Ferry (1963), the close arterial injection of acetylcholine (5–200 μg) evoked a brisk, transient, centripetal asynchronous discharge in fine filaments of the divided splenic nerve. This centripetal activity reduced the height of the C fibre centrifugal maximum compound action potential evoked by stimulating the splenic nerves proximal to the recording electrodes. The acetylcholine-induced discharge in the C fibres was accompanied by the release of noradrenaline and contraction of the capsular smooth muscle of the spleen.

Hexamethonium (5 mg/kg) given intravenously blocked all these effects of acetylcholine but left the effects of electrical stimulation of the splenic nerves unchanged.

Hertting & Widhalm (1965) and Fischer, Weise & Kopin (1966) have reported that in the cat spleen perfused with Krebs solution bretylium blocked the release of noradrenaline in response to electrical stimulation of the splenic nerves at dose levels that did not alter the release of noradrenaline by acetylcholine.

In our experiments, bretylium (0·5–1·0 mg) given close arterially blocked the output of noradrenaline and contractions of the spleen that occurred in response to nerve stimulation (30 c/s) but had much less effect on the responses to acetylcholine (100–200 μg) and to nerve stimulation at 10 c/s.

Bretylium (2–4 mg), however, completely blocked the output of noradrenaline and contractions of the spleen induced by acetylcholine (100–200 μg). These larger doses of bretylium were also required to block the effects of nerve stimulation at 10 c/s. Thus the difference in the sensitivity to blockade of nerve stimulation and acetylcholine was no longer apparent. The outputs of noradrenaline and contractions of the capsular smooth muscle to both nerve stimulation and acetylcholine were partially restored by the close arterial injection of (+)-amphetamine sulphate (100 μg).

Even when the output of noradrenaline in response to acetylcholine had been abolished, acetylcholine still evoked a brisk asynchronous centripetal discharge in the splenic nerves

although a transient reduction in both the height of the evoked centrifugal compound action potential and the asynchronous discharge was seen immediately after bretylium.

The reported differences between the antagonism by bretylium of the effects of acetylcholine and nerve stimulation would therefore seem to be a consequence of bretylium being more effective at blocking high rates of stimulation than low rates of stimulation (Boura & Green, 1962), and not to bretylium blocking the sympathetic nerve at a site proximal to the site of action of acetylcholine in releasing noradrenaline.

REFERENCES

ABERCROMBIE, G. F., DAVIES, B. N. & DWYER, R. A. (1962). The isolated perfused cat's spleen; the measurement of volume changes and of the output of the sympathetic transmitter. *J. Physiol., Lond.*, **161**, 3.

BOURA, A. L. A. & GREEN, A. F. (1962). Comparison of bretylium and guanethidine tolerance, and effects on adrenergic nerve function and responses to sympathomimetic amines. *Br. J. Pharmac. Chemother.*, **19**, 13–41.

BROWN, G. L. & GILLESPIE, J. S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol., Lond.*, **138**, 81–102.

FERRY, C. B. (1963). The sympathomimetic effect of acetylcholine on the spleen of the cat. *J. Physiol., Lond.*, **167**, 487–504.

FISCHER, J. E., WEISE, V. K. & KOPIN, I. J. (1966). Interactions of bretylium and acetylcholine at sympathetic nerve endings. *J. Pharmac. exp. Ther.*, **153**, 523–529.

HERTTING, G. & WIDHALM, S. (1965). Uber den mechanismus der Noradrenalin-Freisetzung aus sympathischen Nervenendigungen. *Arch. exp. Path. Pharmak.*, **250**, 257–258.

Effects of noradrenaline, adrenaline and angiotensin on vascular and capsular smooth muscle of the spleen of the dog

By B. N. DAVIES, J. GAMBLE* and P. G. WITHRINGTON, *Department of Physiology, St. Bartholomew's Hospital Medical College, Charterhouse Square, E.C.*1

The frequency-response characteristics of the vascular and capsular smooth muscle components of the dog's spleen to sympathetic nerve stimulation are different (Davies, Gamble & Withrington, unpublished work). Low frequencies (0·1–1·0/sec) initiate volume changes only while high frequencies (1·0–10/sec) also induce changes in splenic vascular resistance. To determine whether this separation reflects basic differences in the sensitivity of the two types of smooth muscle to the transmitter noradrenaline or intrinsic variations in the innervation we have studied the responses to arterial infusions of noradrenaline and to two other naturally occurring substances, adrenaline and angiotensin.

The preparation has been described elsewhere (Davies & Withrington, 1968). Briefly the spleen of one dog is perfused with blood via the femoral circulation of a second dog. In the present series of experiments a coil of 75 ml. capacity was incorporated in the arterial side.

Arterial perfusion pressure, blood flow, splenic venous pressure and changes in splenic volume were recorded. Changes in splenic vascular resistance were calculated from the changes in arterial pressure and blood flow. Drugs were infused into the proximal part of the splenic arterial tubing at different known rates from which the blood concentration could be calculated. The plateau responses obtained with each infusion were expressed as a percentage of the maximum obtained with each drug. Six to nine concentrations were investigated for each substance so that a major part of the dose-response curve was determined.

In five experiments in which both adrenaline and noradrenaline were infused there was a difference in the dose-response curves of the two smooth muscle systems. For example, perfusion of the spleen with arterial blood containing adrenaline (0·05 μg/ml.) caused a large volume reduction (80% of maximum possible) and a small increase in splenic vascular resistance (16% of maximum possible). Similarly, at low blood concentrations of noradrenaline (0·05 μg/ml.) there was a large reduction in spleen volume (mean 72% max), while the concomitant increase in splenic vascular resistance was only 4% of the maximum. At all concentrations adrenaline was more potent in inducing changes in splenic vascular resistance and volume than noradrenaline.

The action of angiotensin was investigated in three of the above experiments. In contrast to adrenaline and noradrenaline, low blood concentrations—for example, 0·05 μg/ml. of angiotensin—induced profound increases in splenic vascular resistance (mean 85% max) accompanied by very small changes in total spleen volume.

This work was supported by a grant from the Medical Research Council.

REFERENCE

DAVIES, B. N. & WITHRINGTON, P. G. (1968). The effects of prostaglandins E_1 and E_2 on smooth muscle of dog spleen and on its responses to catecholamines, angiotensin and nerve stimulation. *Br. J. Pharmac. Chemother.*, **32**, 136–144.

Prostaglandin output from the spleen

By N. GILMORE, J. R. VANE and J. H. WYLLIE*, *Departments of Pharmacology and Biochemistry, Institute of Basic Medical Sciences, Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2*

Stimulation of the splenic nerve in the dog induces a release of prostaglandins into the venous blood (Davies, Horton & Withrington, 1966). These do not come from the stimulated nerves because (1) phenoxybenzamine and phentolamine, which block the effects of catecholamines on the spleen, also abolish the release of prostaglandins after nerve stimulation (Davies, Horton & Withrington, 1966; Ferreira & Vane, 1967) and (2) prostaglandins are released when the spleen is made to contract by adrenaline (Ferreira & Vane, 1967). During contraction the spleen expels blood which may have a haematocrit as high as 90%, so the prostaglandins might be derived either from the smooth muscle cell, or from the red cells or fluid expelled from the sinusoids of the spleen.

Dog isolated spleens were perfused at constant flow with Krebs solution containing 2% dextran (molecular weight, 110,000). Isolated assay tissues were superfused with the effluent from the spleen to detect release of catecholamines and prostaglandins. Samples of effluent were also assayed for prostaglandins after an extraction procedure designed to remove other pharmacologically active substances.

Stimulation of the splenic nerve at 0·5–50 shocks/sec led to a contraction of the spleen and to an output of blood into the perfusion effluent. At the faster rates of stimulation high concentrations of prostaglandins (10–500 ng/ml. when assayed as E_2) were detected in the effluent. When the stimulation was stopped the spleen returned to its previous volume and the output of prostaglandins ceased. Repeated bursts of nerve stimulation, each

followed by a period of rest, led to a filling of the sinusoids with Krebs solution rather than with blood. At this stage, the spleen still contracted when the nerve was stimulated; the prostaglandins were still released into the effluent, but no more blood was expelled. This showed that prostaglandins were not derived from cells stored in the spleen.

At low rates of stimulation at the beginning of an experiment, there was a large output of blood but no measurable output of prostaglandins. This showed that the prostaglandins were not added to the blood during its storage in the sinusoids. Thus prostaglandins are either liberated when splenic muscle contracts or there is some other tissue in the spleen which secretes prostaglandins in response to nerve stimulation or to catecholamines.

Supported by grants from the Medical Research Council and the Wolfson Foundation.

REFERENCES

DAVIES, B. N., HORTON, E. W. & WITHRINGTON, P. G. (1966). The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. *J. Physiol., Lond.*, **188**, 38–39*P*.

FERREIRA, S. H. & VANE, J. R. (1967). Prostaglandins: their disappearance from and release into the circulation. *Nature, Lond.*, **216**, 868–873.

The ability of indomethacin to antagonize the effect of vasoactive substances on the perfused hindquarters of the guinea-pig

By B. J. NORTHOVER and I. S. RICHARDS*, *Leicester School of Pharmacy, Leicester*

The hindquarters of the guinea-pig were perfused at constant pressure with a modified Tyrode solution containing 2% (w/v) gelatin and ox washed red cells to give a haematocrit of 2·5% (v/v). The weight of the preparation and the rate of flow through it was recorded. Bradykinin (0·5–10 μg), histamine (5–50 μg) and to a small extent 5-hydroxytryptamine caused an initial brief loss in weight of the hindquarters and a simultaneous reduction in flow of perfusate. This was followed by a slow return of rate of flow and of weight towards pre-injection values. The weight gain was caused partly by the expansion of intravascular volume and partly by an accumulation of interstitial (oedema) fluid. A method has been developed for measuring these two components separately. Adrenaline and noradrenaline (0·5–10 μg) caused a reduction in the rate of flow of perfusate which resembled that of histamine and bradykinin, but failed to cause an accumulation of oedema fluid. The flow changes caused by the above vasoactive substances, and the oedema caused by bradykinin and histamine, were antagonized by indomethacin (20–130 μg/ml.).

The effect of drugs on the constriction in response to calcium of isolated depolarized blood vessels

By B. J. NORTHOVER, *Leicester School of Pharmacy, Leicester*

The rat isolated mesenteric artery was perfused at constant rate with a calcium-free potassium-rich ("depolarizing") solution and the perfusion pressure was recorded with a water manometer. When the perfusate was changed to one containing calcium, the artery constricted. This constrictor response was completely inhibited by certain anti-inflammatory

drugs of the analgesic-antipyretic type, such as indomethacin (41 μg/ml.) and flufenamate (12 μg/ml.) by local anaesthetics such as cinchocaine (22 μg/ml.), by spasmolytic drugs such as papaverine (9 μg/ml.) and by desipramine (1·1 μg/ml.).

The constrictor response of "depolarized" smooth muscle to calcium is thought to be caused by the entry of calcium ions into the muscle fibre. The effects of drugs which inhibit the contrictor response to calcium were therefore studied on the entry of radioactive calcium into vascular smooth muscle.

Segments of rat aorta were bathed in calcium-free "depolarizing" solution. They were then transferred to a "depolarizing" solution containing calcium labelled with ^{45}Ca. The uptake of calcium was measured by ashing the muscle, digesting the ash and measuring the radioactivity in the digest by scintillation counting. Local anaesthetics such as cinchocaine reduced the accumulation of radioactive calcium in the muscle in concentrations similar to those required to inhibit the constrictor response to calcium. This suggests that local anaesthetics prevent vascular constriction in response to calcium by preventing the entry of calcium into the muscle fibre. Some of the other drugs which prevent the constrictor response to calcium, such as desipramine, did not, however, reduce the entry of calcium.

Monoamine oxidase activity of platelets

By NOELINE LATT, J. J. RIPPEY* and R. S. STACEY, *Departments of Pathology and of Pharmacology and Therapeutics, St. Thomas's Hospital Medical School, London, S.E.*1

When tryptamine is incubated with platelet-rich plasma, tryptophol and indolylacetic acid are formed and can be separated by acidification of the incubate and extraction into ether. This has been used as the basis for the estimation of monoamine oxidase activity in human and animal platelets. Tryptamine labelled with tryptamine-2-^{14}C bisuccinate was added to platelet-rich plasma to a final concentration of $0{\cdot}2\times10^{-3}$ M and incubated for 30 min alone and in the presence of 10^{-3} M iproniazid in an atmosphere of 5% CO_2 in oxygen. Platelet-poor plasma was treated similarly. The ether soluble metabolites were extracted and the radioactivity of the extracts measured in a liquid scintillation spectrometer. From the results the monoamine oxidase activity of platelets and plasma were calculated.

Under the conditions of the experiment human platelets are able to metabolize in 30 min $0{\cdot}17\pm0{\cdot}01$ (S.E.M.) mg of tryptamine/ml. of packed platelets. Rat and dog platelets have almost no monoamine oxidase activity and the platelets of the guinea-pig and rabbit are considerably less active than human platelets.

Platelets from patients with various blood diseases and during the course of drug administration have also been investigated. The monoamine oxidase activity of the platelets from patients with megaloblastic anaemias caused by untreated vitamin B_{12} or folic acid deficiency was found to be considerably raised ($0{\cdot}49\pm0{\cdot}07$ mg of tryptamine/ml. of packed platelets). Treatment for the deficiency was followed by a decline in platelet monoamine oxidase to normal in 2–3 weeks.

The activation of histidine decarboxylase activity in rat stomach

By Y. KOBAYASHI and D. V. MAUDSLEY* (introduced by G. B. WEST), *Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, U.S.A.*

There are several factors which influence the histidine decarboxylase activity in the gastrie mucosa of the rat. The increase in histidine decarboxylase activity produced by insulin (2 i.u./kg) is reduced or abolished by atropine (1 mg/kg) or bilateral gastric vagotomy whereas the increase produced by pentagastrin (25 μg/kg s.c.) is not affected. Antigastrin (SC 15396), however, does not prevent the increase in histidine decarboxylase activity produced by pentagastrin in doses (25 mg/kg) sufficient to block the gastric acid response. In high doses (100 mg/kg) antigastrin alone produces a slight but significant elevation of histidine decarboxylase activity.

Cycloheximide (10 mg/kg) prevents the increase in histidine decarboxylase activity produced by refeeding, insulin or pentagastrin which suggests that new synthesis of protein is involved. In contrast, actinomycin D (1 mg/kg) does not affect enzyme activation so the synthesis of new enzyme does not seem to depend entirely on DNA primed RNA synthesis. Cycloheximide also produces a rapid and marked reduction in histidine decarboxylase activity in normal animals and calculations on the turnover of the enzyme give a half life of about 100 min. This value agrees with that obtained from following the decay in enzyme activity when animals are starved. In view of the short half life of histidine decarboxylase a drug-induced reduction in enzyme activity may be caused by inhibition of activity of existing enzyme *per se* or by inhibition of the synthesis of new enzyme.

Combination of barbiturates with aldolase

By ANNETTE SKINNER, R. G. SPECTOR* and D. C. WATTS, *Departments of Pharmacology and of Chemistry, Guy's Hospital Medical School, London, S.E.*1

The interaction between barbiturates and enzyme systems is of interest in that it may indicate possible methods of action of this group of drugs. This communication describes a preliminary study of the interaction between barbiturates and aldolase using spectrophotometric techniques. Aldolase was prepared from rabbit muscle and partially purified using an ammonium sulphate solubility method. High concentrations of sodium amylobarbitone, thiopentone sodium, barbituric acid or thiobarbituric acid inhibited the *in vitro* activity of the aldolase enzyme. To investigate the possible mechanism of enzyme inactivation, difference spectra of the barbiturates, enzyme and fructose 1–6 diphosphate were obtained. Evidence of barbiturate and enzyme interaction were obtained with sodium amylobarbitone, thiopentone sodium and barbituric acid but no difference spectrum was seen with thiobarbituric and aldolase.

Interaction of propranolol and neuromuscular blocking agents

By I. ROSENBLUM† and L. WISLICKI*, *Department of Pharmacology, Hebrew University—Hadassah Medical School, Jerusalem, Israel*

Intra-arterial injections of 10 mg or more of propranolol depressed the contraction of skeletal muscles and intensified the action of both depolarizing and non-depolarizing

neuromuscular blocking agents (Wislicki & Rosenblum, 1967). We have now tested the effect of intravenous propranolol infusions on suxamethonium, decamethonium and (+)-tubocurarine, also given intravenously.

Cats were anaesthetized with a urethane–chloralose mixture. The anterior tibialis and soleus muscles were stimulated indirectly by supramaximal square wave pulses to the nerve at a rate of 6/min and contractions were recorded with transducers; carotid blood pressure and heart rate were also monitored. When the preparation had recovered from a partial block produced by a test dose of a curarizing drug, infusions of propranolol (10–60 μg/kg/min) were started; by themselves these did not influence twitch height but soon slowed the heart rate. When bradycardia had become marked the dose of the same neuromuscular blocking drug was repeated while the infusion continued. This resulted in augmentation and prolongation of the block with suxamethonium and decamethonium and in a diminution of the blocking action of (+)-tubocurarine, as compared with the effect before propranolol.

The difference in the effect of propranolol on the action of depolarizing and non-depolarizing blocking agents may be the result of a sympathomimetic action of the adrenaline β-receptor blocking agent because propranolol has been reported to release catecholamines (Kayaalp & Kiran, 1966) which in turn may augment the release of acetylcholine (Krnjevic & Miledi, 1958). An increased availability of transmitter at the neuromuscular junction during intravenous infusion of propranolol would result in an intensification of the blocking effect of depolarizing drugs but it would antagonize tubocurarine (Hutter & Loewenstein, 1955; Bowman, Goldberg & Raper, 1962). Experiments to test this hypothesis by electrophysiological methods are in hand.

† Public Health Service research career programme awardee, 5-K3-HE-25, 420-02, National Heart Institute. Present address: Department of Pharmacology, Albany Medical College, Albany, N.Y., U.S.A.

REFERENCES

BOWMAN, W. C., GOLDBERG, A. A. J. & RAPER, C. (1962). A comparison between the effects of a tetanus and the effects of sympathomimetic amines on fast- and slow-contracting mammalian muscles. *Br. J. Pharmac. Chemother.*, **19**, 464–484.

HUTTER, O. F. & LOEWENSTEIN, W. R. (1955). Nature of neuromuscular facilitation by sympathomimetic stimulation in frog. *J. Physiol., Lond.*, **130**, 559–571.

KAYAALP, S. O. & KIRAN, B. K. (1966). Mechanism of a sympathomimetic action of propranolol in dog. *Br. J. Pharmac. Chemother.*, **28**, 15–22.

KRNJEVIC, K. & MILEDI, R. (1958). Some effects produced by adrenaline upon neuromuscular propagation in rats. *J. Physiol., Lond.*, **141**, 291–304.

WISLICKI, L. & ROSENBLUM, I. (1967). The effects of propranolol on normal and denervated muscle. *Archs int. Pharmacodyn. Thér.*, **170**, 117–123.

A comparison of the blocking properties of propranolol and MJ. 1999 at β-receptors and of their effects in hypertensive animals

By J. B. FARMER and G. P. LEVY*, *Pharmacology Department, Allen and Hanburys Limited, Ware, Hertfordshire*

Propranolol, which antagonizes the effects of catecholamines at β-receptors, has been reported to lower blood pressure in hypertensive subjects. The effect of propranolol and

the β-receptor antagonist MJ. 1999 [4-(2-isopropylamino-1-hydroxyethyl)-methanesulphonanilide hydrochloride] have been examined in normotensive rats and dogs and metacorticoid hypertensive rats and renal hypertensive dogs.

In anaesthetized rats and dogs the β-receptor blocking activity of MJ. 1999 (1–3 mg/kg I.V. or S.C.) is approximately one-third that of propranolol (0·4–0·8 mg/kg), whilst orally in conscious dogs MJ. 1999 (1 mg/kg) is approximately half as potent as propranolol. In contrast to propranolol, MJ. 1999 has neither quinidine-like effects on the heart nor local anaesthetic activity. Propranolol (1–20 mg/kg) and MJ. 1999 (2–60 mg/kg) given subcutaneously to conscious hypertensive rats produced bradycardia and at high dose levels propranolol (50 mg/kg) but not MJ. 1999 (150 mg/kg) produced a fall in blood pressure together with marked reduction in pulse pressure. At this dose level propranolol but not MJ. 1999 produced changes in the e.c.g. of normotensive rats resembling those produced by quinidine (50 mg/kg). When given orally to conscious hypertensive dogs in doses (20 mg/kg) twenty times greater than that required to produce β-receptor blockade propranolol caused a small bradycardia but had no effect on systolic blood pressure. Even daily oral administration of propranolol (5 mg/kg) to conscious hypertensive dogs for 2 months at five times the dose necessary for β-receptor block failed to lower systolic blood pressure although heart rate was reduced.

These results indicate that effective β-receptor blockade does not lead to a fall in blood pressure in conscious hypertensive rats or dogs.

The lack of influence of adrenergic nerves on physiological tremor in man

By R. D. LOWE, C. D. MARSDEN and J. C. MEADOWS* (introduced by R. S. STACEY), *Department of Medicine, St. Thomas's Hospital Medical School, London, S.E.*1

Infusions of adrenaline or isoprenaline cause an increase of tremor of the outstretched hand which results from local activation of β-receptors for catecholamines in the forearm (Marsden, Foley, Owen & McAllister, 1967). Because noradrenaline, in addition to its powerful α-effects, has some effect on β-receptors, we have examined the possibility that adrenergic nerves could influence physiological tremor. Tremor was recorded from an accelerometer strapped to the outstretched fingers and forearm blood flow was measured by plethysmography, using a mercury-in-rubber strain gauge.

Intra-arterial infusions of noradrenaline (0·1–1·0 μg/min) had no effect on tremor, but after treatment of the forearm with intra-arterial phenoxybenzamine (5 mg) the same dose of noradrenaline usually caused an increase of tremor. This finding suggests that infused noradrenaline can stimulate the β-receptors responsible for an increase of tremor, but that concomitant stimulation of α-receptors can mask this effect.

Because noradrenaline infusions do not necessarily reproduce the effects of sympathetic nerve stimulation, we attempted to elicit local release of noradrenaline from sympathetic nerve terminals, by infusing tyramine or by reflex activation of the sympathetic. Intra-arterial infusions of tyramine (50–800 μg/min) caused no increase of tremor either before or after phenoxybenzamine although these doses had effects on blood flow comparable with those of noradrenaline.

The sympathetic nerves to the forearm were stimulated reflexly by lower body suction (Brown, Goei, Greenfield & Plassaras, 1966) applied up to the hips, using negative pressures of 25–90 mm Hg; this invariably caused profound vasoconstriction in the forearm, but rarely caused an increase of tremor (three out of fourteen occasions). After treatment of the forearm with phenoxybenzamine tremor was more frequently elicited but the increased tremor could not be abolished by intra-arterial bretylium in a dose which blocked the reflex vasoconstriction (12·5 mg).

These results suggest that adrenergic nerves to the forearm do not have an important influence on tremor, perhaps because the β-receptors concerned with tremor are not situated sufficiently close to the sympathetic nerve endings. The occasional increase of tremor caused by lower-body suction is caused by some mechanism other than reflex activation of adrenergic nerves to the forearm, and may well be the result of catecholamines circulating in the blood stream.

REFERENCES

BROWN, E., GOEI, J. S., GREENFIELD, A. D. M. & PLASSARAS, G. C. (1966). Circulatory responses to simulated gravitational shifts of blood in man induced by exposure of the body below the iliac crests to subatmospheric pressure. *J. Physiol., Lond.*, **183**, 607-627.

MARSDEN, C. D., FOLEY, T. H., OWEN, D. A. L. & MCALLISTER, R. G. (1967). Peripheral adrenergic receptors concerned with tremor. *Clin. Sci.*, **33**, 53-65.

Assessment of the relative toxicity of local analgesic drugs in man

By FELICITY REYNOLDS (introduced by R. S. STACEY), *Department of Pharmacology and Therapeutics, St. Thomas's Hospital Medical School, London, S.E.*1

The largest doses of local analgesic drugs used in clinical practice today are usually those given by the epidural route. A solution injected into the epidural space is separated from the spinal roots by their dural coverings, and a relatively large dose is necessary for analgesia. This technique may be used to provide analgesia for surgery, during the post-operative period and during the first and second stages of labour. Repeated doses may be needed. The rate of absorption of a local analgesic from the epidural space, its elimination and placental transfer are important considerations if toxic effects are to be avoided.

A technique for the estimation of the local analgesic drugs lignocaine, mepivacaine (Carbocaine) and bupivacaine (Marcain) in the blood, using gas chromatography, has been devised. Only 2 ml. of blood are necessary, and bupivacaine (0·05 μg/ml.) (for which the method is least sensitive) may be measured, with an accuracy of $\pm 5\%$ at 0·1 μg/ml.

The concentration in the blood of patients undergoing epidural analgesia and in the cord blood of the foetus at delivery has been measured. The rate of disappearance from the blood stream of volunteers given a slug dose of a single drug or of a mixture of drugs has also been investigated.

The calculation of the half-life of a lipid soluble substance presents difficulties, because distribution into the tissues plays a large part in the disappearance of the substance from the blood stream. In some cases a single exponential fall-off is seen within the 2–2·5 hr of measurement, and the half-life of the drug in the body can then be calculated; in others more than one exponential can be detected. Significant differences in the elimination of the

three drugs in one individual are observed, however, and bear out the impression of some clinicians that mepivacaine is likely to accumulate significantly after repeated doses. Of the three drugs investigated bupivacaine appears to be the most rapidly eliminated, and to cross the placenta less than the other two drugs.

I am indebted to Professor A. H. Beckett of the Department of Pharmacy, Chelsea College of Science and Technology, for providing me with advice and facilities to carry out the estimations.

DEMONSTRATIONS

Bretylium-like action of debrisoquin in the human eye

By J. M. SNEDDON and P. TURNER, *Department of Pharmacology and Clinical Pharmacology Division, Medical Professorial Unit, St. Bartholomew's Hospital, London, E.C.*1

Guanethidine and bretylium potentiate the effects of directly acting sympathomimetic amines, but whereas guanethidine abolishes the effect of indirectly acting amines, bretylium does not (Green, 1962). Debrisoquin sulphate, a new antihypertensive agent, resembles bretylium in producing adrenergic nerve blockade without catecholamine depletion (Moe, Bates, Palkoski & Banziger, 1964) and its use in man is associated with exaggerated pressor responses to tyramine and noradrenaline (Abrams, Pocelinko, Klausner, Hanauer & Whitman, 1964).

The acute and chronic administration of local guanethidine in the human eye produces potentiation of the mydriasis following instillation of phenylephrine, adrenaline and methoxamine eye drops and the abolition of the mydriatic activity of indirectly acting amines such as tyramine, ephedrine, amphetamine, hydroxyamphetamine and phenmetrazine (Sneddon & Turner, 1967a, b).

The effects of local administration to the eye of guanethidine and debrisoquin sulphate have been compared in seven thyrotoxic patients with bilateral lid retraction. The method used was that described by Sneddon & Turner (1967a) in which the subjects' eyes were

TABLE 1

INFLUENCE OF LOCAL GUANETHIDINE AND DEBRISOQUIN ON EPHEDRINE AND PHENYLEPHRINE MYDRIASIS IN THE HUMAN EYE

−, No mydriasis. +, 10–30% increase in mydriasis; ++, 30–60% increase in mydriasis; +++, 60+% increase in mydriasis.

			Mydriasis		
			Phenylephrine 5%		
	Miosis	Ephedrine 2%	24 hours	7 days	28 days
No. of patients	7	7	7	3	1
Guanethidine 10%	Present	−	++	+++	+++
Debrisoquin 2%	Absent	+	+	++	+++

photographed under standard lighting conditions and changes in pupil diameter expressed as % increase or decrease over pre-test control values. Standard mydriatic responses to ephedrine 2% and phenylephrine 5% were determined over a 30 min period on two separate occasions before the twice daily instillation of one drop 10% guanethidine into one eye and 2% debrisoquin into the other of each patient. Both eyes were challenged with 5% phenylephrine 24 hr, 7 days and in one subject 28 days after the start of chronic adrenergic neurone block. 2% ephedrine challenge was carried out at 48 hr in all patients and 24 days in one. The results are shown in Table 1, and demonstrate that both drugs produced a potentiated response to phenylephrine. Guanethidine abolished the response to ephedrine, but debrisoquin did not, and in fact showed some potentiation. A marked ephedrine mydriasis was still present after 24 days in the debrisoquin-treated eye of the one patient studied for this period. The slower development of potentiation to phenylephrine by debrisoquin may be because the concentration of this drug was lower compared with guanethidine, and this may also account for the absence of a significant miotic effect of debrisoquin.

These results indicate that debrisoquin has an action in the human eye which resembles that of bretylium in other experimental situations (see Boura & Green, 1959).

We thank the physicians of St. Bartholomew's Hospital who allowed us to study their patients. One of us (J.M.S.) was supported by a research grant from the Board of Governors. Guanethidine and debrisoquin eye drops were kindly supplied by Ciba Laboratories, Ltd., and Roche Products, Ltd., respectively.

REFERENCES

ABRAMS, W. B., POCELINKO, R., KLAUSNER, M., HANAUER, L. & WHITMAN, E. N. (1964). Clinical pharmacological studies with debrisoquin sulphate, a new hypertensive agent. *J. new Drugs*, **4**, 268–283.

BOURA, A. L. A. & GREEN, A. F. (1959). The action of bretylium: adrenergic neurone blocking and other effects. *Br. J. Pharmac. Chemother.*, **14**, 536–548.

GREEN, A. F. (1962). Antihypertensive drugs. *Advances in Pharmacology*, ed. Garattini, S. & Shore, P. A., vol. 1, pp. 162-220. London: Academic Press.

MOE, R. A., BATES, H. M., PALKOSKI, Z. M. & BANZIGER, R. (1964). Cardiovascular effects of 3,4-dihydro-2 (1H) isoquinoline carboxamidine (Declinax). *Curr. Ther. Res.*, **6**, 299.

SNEDDON, J. M. & TURNER, P. (1967a). The effect of local guanethidine on the palpebral fissure and the pupil in thyrotoxicosis, and its interaction with sympathomimetic amines. *J. Physiol., Lond.*, **189**, 20–22*P*.

SNEDDON, J. M. & TURNER, P. (1967b). Structure-activity relationship of some sympathomimetic amines in the guanethidine-treated human eye. *J. Physiol., Lond.*, **192**, 23–26*P*.

Behaviour of mice after administration of an amphetamine-barbiturate mixture (16 mm film)

By DAPHNÉ JOYCE, R. D. PORSOLT, HANNAH STEINBERG and A. SUMMERFIELD, *Department of Psychology, Birkbeck College, University of London, London, W.C.*1

A mouse placed on a horizontal wooden board (41 × 41 cm) pierced with sixteen holes (3·1 cm diameter and 1·8 cm deep) engages in locomotor activity across the board and also plunges its head into the holes (Boissier & Simon, 1964). The number of holes visited during a specified interval of time can be counted. If several observers take part in an experiment, each watching one mouse at a time, it is possible to carry out extensive dose-response studies with drugs on a single day and with animals which have the same past experience.

This method has been used to test the generality of the effect of an amphetamine-barbiturate mixture. Rushton & Steinberg (1963) found that, after administration of a mixture of amphetamine sulphate (0·75 mg/kg) and amylobarbitone sodium (15·0 mg/kg) to rats, the locomotor activity in a Y-maze increased much more than after the same dose of the drugs administered alone or in any other combination.

Dose-response curves were obtained for different doses of amphetamine and amylobarbitone (in a mixture of constant ratio of 1:20) and for the single drugs by scoring the number of head entries of mice into the holes of the board. As in the case of results from the Y-maze test with rats, an inverted V dose-response relation was obtained for the mixture. The maximum increase in head entries was recorded for a dose of the mixture containing amphetamine sulphate (1·5 mg/kg) and amylobarbitone sodium (30·0 mg/kg).

REFERENCES

BOISSIER, J. R. & SIMON, P. (1964). Dissociation de deux composantes dans le comportement d'investigation de la souris. *Archs int. Pharmacodyn. Thér.*, **147**, 372–387.

RUSHTON, R. & STEINBERG, H. (1963). Dose-response relations of amphetamine-barbiturate mixtures. *Nature, Lond.*, **197**, 1017–1018.

A method of recording isotonic contraction of isolated tracheobronchial muscle

By A. H. CHRISTIANSON and W. J. F. SWEATMAN (introduced by H. O. J. COLLIER), *Department of Pharmacological Research, Parke-Davis and Co., Hounslow, Middlesex*

Various methods of preparation and recording have been used to study the action of drugs on isolated tracheobronchial muscle (Castillo & De Beer, 1947; Rosa & McDowall, 1951; Akçasu, 1952; Foster, 1966) and electronic instruments for recording isotonic

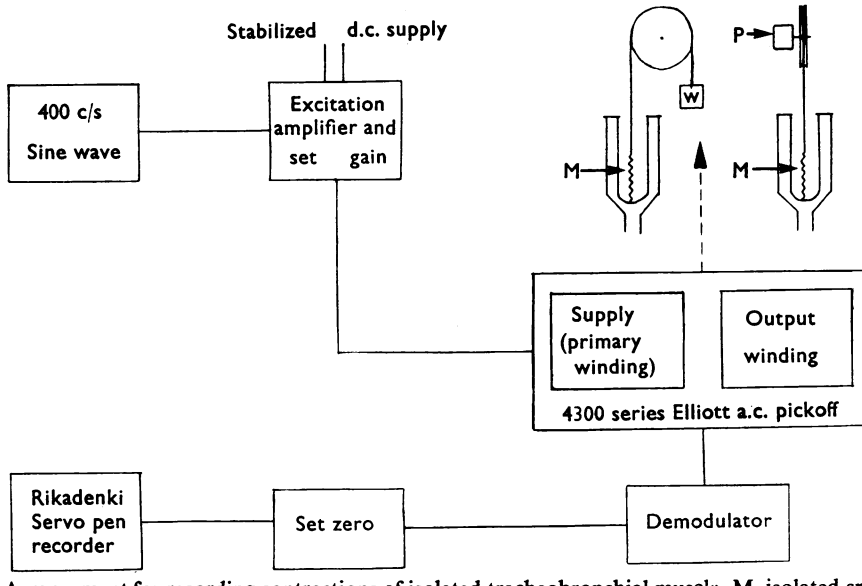

Fig. 1. Arrangement for recording contractions of isolated tracheobronchial muscle. M, isolated spirally-cut muscle; P, a.c. pick-off attached coaxially to pulley wheel; W, 250 mg weight.

contractions have been described (Wilkie, 1956; Erdös, Jackman & Barnes, 1962). The apparatus to be demonstrated is believed to be more accurate, more convenient and more sensitively adjustable than those previously described.

A nylon suture (Arbralon size 0) is attached at one end to the isolated muscle and at the other to a 250 mg weight (Fig. 1). It is looped over an aluminium pulley wheel (diameter 8 cm), which is attached coaxially to the spindle of an alternating current pick-off type 4300-3b-1 (Elliott). The pick-off acts as a variable rotary transformer with a linear relationship between output voltage and displacement of the rotor. The output voltage is recorded by a self-balancing servo-potentiometer (Rikadenki).

In setting up, the output signal after demodulation is backed off so that the signal measured by the potentiometer is adjustable about zero voltage. Linearity is checked using a micrometer giving equal steps. The linearity is restricted to a 30° angle of rotation, but is found to be adequate for tracheobronchial preparations.

One advantage of this apparatus over the linear variable differential transformer lies in the abolition of a second pulley wheel leading to less friction and greater simplicity. Another advantage is that the tension exerted on the muscle is constant throughout contraction, unlike many previously described "isotonic" systems. This system has enabled us to record spontaneous rhythmical contractions and relaxations of human bronchial muscle *in vitro*.

The method is demonstrated using guinea-pig isolated trachea prepared as described by Bhoola, Collier, Schachter & Shorley (1962) and human isolated bronchus as described by Rosa & McDowall (1951).

REFERENCES

AKÇASU, A. (1952). The actions of drugs on the isolated trachea. *J. Pharm. Pharmac.*, **4**, 671.

BHOOLA, K. D., COLLIER, H. O. J., SCHACHTER, M. & SHORLEY, P. G. (1962). Actions of some peptides on bronchial muscle. *Br. J. Pharmac. Chemother.*, **19**, 190–197.

CASTILLO, J. C. & DE BEER, E. J. (1947). The tracheal chain; a preparation for the study of antispasmodics with particular reference to bronchodilator drugs. *J. Pharmac. exp. Ther.*, **90**, 104–109.

ERDÖS, E. G., JACKMAN, V. & BARNES, W. C. (1962). Instrument for recording isotonic contractions of smooth muscle. *J. appl. Physiol.*, **17**, 367–368.

FOSTER, R. W. (1966). The nature of adrenergic receptors of the trachea of the guinea-pig. *J. Pharm. Pharmac.*, **18**, 1–12.

ROSA, L. M. & MCDOWALL, R. J. S. (1951). The action of the local hormones on the isolated human bronchus. *Acta allerg. kbh.*, **4**, 293–304.

WILKIE, D. R. (1956). The mechanical properties of muscle. *Brit. med. Bull.*, **12**, No. 3, 177–182.

A new stimulator

By P. M. G. BELL (introduced by J. R. VANE), *University Laboratory of Physiology, Oxford*

Stimulators able to deliver high currents are needed in experiments in which isolated tissues are excited in large volumes of highly conducting fluid.

A stimulator meeting these requirements is now demonstrated. It consists of a number of circuit modules compatible with those previously described (Bell, 1966) for counting and gating trains of impulses. The stimulator can be used to provide either impulses at constant frequency or a regular cycle consisting of a train of impulses repeated at fixed intervals of time.

The specification of this instrument is:

Cycle duration:	1 msec to 1,000 sec
Delay:	1 msec to 10 sec
Duration of train of pulses:	10 msec to 100 sec
Frequency of pulses in train:	1/10 sec to 1,000/sec
Pulse duration:	100 μsec to 10 sec
Pulse amplitude:	0 to 25 V at 500 mA
Output impedance:	2 Ω.

REFERENCE

BELL, P. M. G. (1966). Transistorized modular circuit elements. *J. Physiol., Lond.*, **186**, 15–16*P*.

Effects of salicylates on temperature regulation in man

By W. I. CRANSTON and C. ROSENDORFF (introduced by R. S. STACEY), *Department of Medicine, St. Thomas's Hospital Medical School, London, S.E.*1

Good evidence exists that salicylates can reduce a febrile body temperature, but there are conflicting reports on their effects on normal temperature. Salicylates have anti-inflammatory effects (Smith, 1966), and it is not clear whether their antipyretic properties are a consequence of these, or whether they result from a direct action on thermoregulation.

The effects of salicylates on normal thermoregulation have been examined by measuring the responses of oral and ear temperature and finger heat elimination to known thermal loads in normal volunteers. Oral salicylate was administered as three doses of soluble aspirin (1·2 g) at hourly intervals, and measurements were made between 90 and 200 min after the last dose. Plasma salicylate levels averaged 21·8 mg/100 ml. At this salicylate level, there was no change either in resting temperature or in the sensitivity of the thermoregulatory system to applied heat loads.

The time of onset of the effect of sodium salicylate on the body temperature of febrile patients has also been measured: if aspirin antipyresis is a consequence of the anti-inflammatory effects of the drug, it might be expected that it would take some time before any effect is manifest. Sodium salicylate was given as a single intravenous injection of 2 g. followed by a continuous intravenous infusion at 9–15 mg/min, to fifteen patients with febrile illnesses, and twelve afebrile patients as controls. Temperature was continuously recorded from the mouth and/or external auditory meatus, and finger heat elimination was followed in several patients. In afebrile controls, there was no change in temperature or heat elimination. In febrile patients, body temperature showed a significant fall, beginning within 5 min of the start of the infusion. This was accompanied by increased heat elimination.

It is concluded that salicylates have no detectable effect on normal thermoregulation, but that they have a very rapid effect in fever caused by disease. This suggests that they interfere specifically with the process whereby fever is induced and implies that they have some influence upon the effects of endogenous pyrogen in the hypothalamus.

REFERENCE

SMITH, M. J. H. (1966). Anti-inflammatory activity of salicylates. In SMITH, M. J. H. & SMITH, P. K. *The Salicylates; a Critical Bibliographic Review*. New York: Interscience.

Hypothalamic blood flow in conscious rabbits; effects of monoamines and pyrogen

By W. I. CRANSTON and C. ROSENDORFF (introduced by R. S. STACEY), *Department of Medicine, St. Thomas's Hospital Medical School, London, S.E.*1

The monoamines noradrenaline and 5-hydroxytryptamine affect body temperature when injected into the lateral ventricles or hypothalamus of several species (Cranston & Rosendorff, 1967; Feldberg, Hellon & Lotti, 1967), including the rabbit (Cooper, Cranston & Honour, 1965). One way in which these temperature changes may be induced is by an alteration in blood flow, and hence local temperature, in the hypothalamus.

By observing the rate at which the inert isotope ^{133}Xe is cleared from the site of injection, an estimate of local blood flow is obtained. This method has been used in skeletal muscle, where its validity has been demonstrated with simultaneous venous occlusion plethysmography (Lassen, Lindbjerg & Dahn, 1965). We have adapted the ^{133}Xe clearance technique to assess local hypothalamic blood flow in the conscious rabbit. Using a modification of the stereotaxic technique described by Monnier & Gangloff (1961), a fine cannula was fixed with its tip in the hypothalamus. Volumes of 2·5–10 μl. of a saline solution of ^{133}Xe containing 5 mc/ml. were injected, and the γ emission was measured by an external collimated scintillation probe and ratemeter, and recorded continuously on an ultra-violet recorder. Injections were repeated at intervals of 15–30 min. Clearance curves were mono-exponential, and the half decay times varied from 1·00 to 2·05 min in control animals. The half times ($T_{\frac{1}{2}}$) were converted to nominal blood flow rates, using the formula: blood flow (ml./g tissue/min) $= \frac{0{\cdot}693\lambda}{T_{\frac{1}{2}}}$, where $\lambda = 0{\cdot}63$, the tissue:blood partition coefficient for rabbit cerebrum (Andersen & Ladefoged, 1967).

In control experiments, when intravenous or intraventricular injections of normal saline were given, the hypothalamic flow rates rose by an average of 19% over 2 hr. Inhalation of 4% CO_2 in air caused a rapid increase of flow, to levels 38% above the control values.

Intravenous injection of 1 μg endotoxin ("E" pyrogen, Organon Laboratories, Ltd.) caused an increase in hypothalamic flow, which was significantly greater than the small rise seen in control experiments.

Intraventricular injection of noradrenaline (20 μg) caused a rise in hypothalamic blood flow in most, but not all, experiments, and usually a rise in body temperature. Intraventricular injection of 5-hydroxytryptamine (200 μg) caused an inconsistent decrease in hypothalamic flow, and usually a decrease in body temperature.

In two animals, body temperature was raised by increasing the environmental temperature. Both showed a rise in hypothalamic flow.

These observations indicate that the clearance of locally injected ^{133}Xe provides a useful index of regional blood flow; and that hypothalamic blood flow changes in response to agents which affect thermoregulation.

REFERENCES

ANDERSEN, A. M. & LADEFOGED, J. (1967). Partition coefficient of ^{133}xenon between various tissues and blood *in vivo*. *Scand. J. clin. Lab. Invest.*, **19**, 72–78.

COOPER, K. E., CRANSTON, W. I. & HONOUR, A. J. (1965). Effects of intraventricular and intrahypothalamic injection of noradrenaline and 5-HT on body temperature in conscious rabbits. *J. Physiol., Lond.*, **181**, 852–864.

CRANSTON, W. I. & ROSENDORFF, C. (1967). Central temperature regulation in the conscious rabbit after monoamine oxidase inhibition. *J. Physiol., Lond.*, **193**, 359–374.

FELDBERG, W., HELLON, R. F. & LOTTI, V. J. (1967). Temperature effects produced in dogs and monkeys by injections of monoamines and related substances into the third ventricle. *J. Physiol., Lond.*, **191**, 501–515.

LASSEN, N. A., LINDBJERG, I. F. & DAHN, I. (1965). Validity of the xenon^{133} method for measurement of muscle blood flow evaluated by simultaneous venous occlusion plethysmography. *Circulation Res.*, **16**, 287–293.

MONNIER, M. & GANGLOFF, H. (1961). *Atlas for Stereotaxic Brain Research on the Conscious Rabbit.* London: Elsevier.

The respiratory stimulant action of salicylates

By I. R. CAMERON (introduced by R. S. STACEY), *Department of Medicine, St. Thomas's Hospital Medical School, London, S.E.*1

Salicylates in both toxic and therapeutic doses cause respiratory stimulation in animals and man. The respiratory response to salicylates is complex, because a central medullary stimulation as well as a peripheral metabolic action contribute to the hyperpnoea. The central and peripheral components have been studied by comparing the effects of injecting salicylate either intravenously or into the cerebral ventricles of conscious rabbits.

To permit subsequent injection into a lateral ventricle each rabbit was fitted with a stereotaxic headplate under pentobarbitone anaesthesia, using the technique of Monnier & Gangloff (1961), as adapted by Cooper, Cranston & Honour (1965). Five to ten days were allowed for the rabbit to recover before it was used for an experiment. During the experiment arterial pCO_2 and pH were measured on samples withdrawn anaerobically from a central ear artery; in four rabbits ventilation was measured after recovery from the anaesthetic necessary for tracheostomy. Sodium salicylate (1·2 mg) dissolved in 0·4 ml. of a mock cerebrospinal fluid was introduced into a lateral ventricle via the headplate cannula. Cerebrospinal fluid was sampled by cisternal puncture performed as soon as possible after the animal had been anaesthetized. 0·7 to 1·0 ml. of cerebrospinal fluid could be withdrawn anaerobically providing sufficient volume for repeated pH determinations.

Injection of sodium salicylate into a lateral ventricle of conscious rabbits always caused a fall in arterial pCO_2 and a rise in arterial pH. Ventilation was measured in four rabbits, and was always found to increase.

The changes in CO_2 production following intravenous sodium salicylate (150 mg/kg body weight) were compared with those occurring after intraventricular administration of salicylate. Following intravenous administration a rise in CO_2 production was observed, whereas after injection into a lateral ventricle there was no change in CO_2 production, confirming the results of Tenney & Miller (1955) on anaesthetized dogs.

It has been suggested (Plum & Swanson, 1963) that the hyperventilation observed in salicylate overdose might be secondary to a cerebrospinal fluid acidosis. The pH of the cerebrospinal fluid was measured in five rabbits 1 hr after intraventricular administration of sodium salicylate and was found to be $7{\cdot}45 \pm 0{\cdot}02$ (mean $\pm$ S.E.M.). In a group of control rabbits the pH of the cerebrospinal fluid was $7{\cdot}31 \pm 0{\cdot}02$.

It is concluded therefore that by injecting sodium salicylate into the cerebral ventricles, the central stimulant action may be investigated separately from the metabolic consequences

of intravenous administration. There is no evidence that the central respiratory stimulation is associated with cerebrospinal fluid acidosis.

REFERENCES

COOPER, K. E., CRANSTON, W. I. & HONOUR, A. J. (1965). Effects of intraventricular and intrahypothalamic injection of noradrenaline and 5-hydroxytryptamine on body temperature in conscious rabbits. *J. Physiol., Lond.*, **181**, 852–864.

MONNIER, M. & GANGLOFF, H. (1961). *Atlas for Stereotaxic Brain Research on the Conscious Rabbit.* London: Elsevier.

PLUM, F. & SWANSON, A. G. (1963). Relationship to hyperpnea of blood and CSF acid-base balance in methanol and salicylate poisoning. *Clin. Res.*, **11**, 120.

TENNEY, S. M. & MILLER, R. M. (1955). The respiratory and circulatory actions of salicylate. *Am. J. Med.*, **19**, 498–508.

Effects of alpha-methyl-*p*-tyrosine in malignant phaeochromocytoma

By N. F. JONES, C. R. J. RUTHVEN, M. SANDLER and G. WALKER, *St. Thomas's Hospital Medical School, and the Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, London*

The subject was a 67-year-old woman who was discovered to have a phaeochromocytoma in May 1966. At that time the pattern of catecholamine excretion in the urine was as follows:

Noradrenaline	2,069 μg/24 hr
Adrenaline	41 μg/24 hr
Dopamine	1,481 μg/24 hr
4-Hydroxy-3-methoxymandelic acid (VMA)	48 mg/24 hr
Homovanillic acid	10 mg/24 hr
Total metadrenalines	7·7 mg/24 hr
4-Hydroxy-3-methoxyphenylglycol	22 mg/24 hr
(all these values are raised)	

The phaeochromocytoma was removed but her symptoms later recurred and in May 1967 she was found to have a recurrence of the tumour at its original site with hepatic metastases.

In May 1967 alpha-methyl-*p*-tyrosine was given in doses of up to 2 g/day. This reduced urinary excretion of VMA from a mean value of 145 mg/day to 20–25 mg/day, and urinary total metadrenalines fell from a mean value of 9·5 mg/day to 1·2–2·8 mg/day.

The main clinical effects of catecholamine excess were extreme sweating, obstinate constipation, tachycardia with multiple extrasystoles, and incapacitating chronic postural hypotension with hypertension in the supine position (190/115 supine; 90/60 erect). At this time the response of the patient's circulation to the Valsalva manœuvre was abnormal and the blood volume was considerably reduced (51·7 ml./kg). Treatment with phenoxybenzamine (up to 200 mg/day) was without apparent effect. Within two weeks of starting therapy with alpha-methyl-*p*-tyrosine all the above symptoms disappeared. In particular the postural hypotension was markedly alleviated. The patient, who previously had been unable to tolerate quiet standing for more than 1–2 min, was now able to walk 300 yards unaided. Total blood volume did not increase after alpha-methyl-*p*-tyrosine therapy, so that the correction of postural hypotension must be ascribed to an alteration in the vasomotor response to posture.

Comparison of the effect of angiotensin and noradrenaline on sodium excretion in the sodium loaded rat

By M. A. BARRACLOUGH, N. F. JONES and C. D. MARSDEN (introduced by R. S. STACEY), *The Medical Unit, St. Thomas's Hospital Medical School, London, S.E.*1

The effects of angiotensin on urinary sodium excretion in the rat depend on the experimental conditions (Barraclough, Perriello, Marsden & Jones, 1967). The antinatriuretic effect of small subpressor doses of angiotensin can only be elicited in chronically sodium loaded rats, habituated to the experimental conditions. We have compared the effect of noradrenaline and angiotensin in those circumstances to see whether noradrenaline might play any part in sodium economy.

The experiments were performed on conscious trained rats weighing about 200 g with indwelling arterial, venous and bladder catheters. They were infused with 0·5 m-equiv of sodium a day for 3 days before the study. During the experiment the animals were continuously infused intravenously with hypotonic saline (40m-equiv/l.) at 0·37 ml./min. Noradrenaline and angiotensin II (Ciba) were infused alternately in the same animal for periods of 10 min.

The effect of each agent on urinary sodium excretion is shown in Table 1. Infusion of angiotensin in doses of 0·00005 and 0·0005 μg/kg/min which had no pressor effect, or in doses of 0·005 μg/kg/min which had a slight pressor effect in some animals, consistently reduced urine flow and urinary sodium concentration and excretion.

TABLE 1

	Noradrenaline		Angiotensin	
Dose μg/kg/min	Number of experiments	Mean sodium excretion (% of control)	Number of experiments	Mean sodium excretion (% of control)
0·00005			2	77·2
0·0005	3	101·4	7	81·9
0·005	10	100·9	7	68·0
0·05	9	103·2	4	76·0
0·25	5	105·8		
0·5	10	124·8	2	148·7
5·0	10	196·3		

Angiotensin in pressor doses of 0·05–0·5 μg/kg/min reduced urine flow and sodium excretion for the first 5 min of infusion. This was followed by an increase in flow and particularly in sodium excretion. This secondary natriuretic effect predominated with the largest dose of 0·5 μg/kg/min. Noradrenaline in subpressor doses of 0·05–0·0005 μg/kg/min had no effect on urine flow or sodium excretion. Noradrenaline in pressor doses of 0·25–5 μg/kg/min increased urine flow and sodium excretion, without any initial reduction as seen with angiotensin. Thus there was no dose of noradrenaline which reduced sodium excretion. It is concluded that circulating noradrenaline has no important effect on the economy of sodium in this animal.

REFERENCE

BARRACLOUGH, M. A., PERRIELLO, V. A., MARSDEN, C. D. & JONES, N. F. (1967). Influence of the experimental conditions on the renal response to angiotensin in the rat. *Experientia*, **23**, 140.