A rapid in vivo test for dependence potential of analgesic drugs

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Collier, Francis, Henderson & Schneider (1974) observed that heroin (1 mg/kg s.c.) reduced the incidence of the quasi-morphine abstinence syndrome (QMAS) produced by giving a single dose of theophylline orally to naive rats. The effect of heroin was removed by the simultaneous administration of naloxone (0.2 mg/kg). More recently, Collier & Francis have shown that whereas levorphanol reduced the quasi-morphine abstinence effect induced by another methylxanthine, 3-isobutyl-1-methylxanthine (IBMX) its stereoisomer dextrorphan had no effect. These results suggested therefore that suppression by narcotic drugs of the QMAS could be used as a rapid *in vivo* method for assessing dependence potential, in a similar way to the conventional single dose suppression test, an example of which was described by Collier & Schneider (1972). Of particular interest in the present series of experiments were the actions of several orally effective drugs commonly used to relieve pain in man, that had differing dependence potential but similar analgesic potency.

The dose-response line for pentazocine suppressing QMAS was shallower that that of codeine, *d*-propoxyphene or pethidine. Rats given an opiate with IBMX and challenged 30 min later by naloxone (0.3 mg/kg) showed a significant dose-related increase in withdrawal jumping, compared with rats given an opiate or IBMX alone. Thus, the physical dependence potential of an opiate may be due to its reducing indirectly, cyclic AMP phosphodiesterase activity in morphine-sensitive neurones.

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Sympathetic nerve recording in the conscious rabbit

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The recording of electrical activity from peripheral sympathetic nerves is a useful technique for the evaluation of centrally acting hypotensive drugs. Records of pre- or postganglionic sympathetic activity can be obtained by placing the splanchnic or renal nerve on bipolar electrodes and covering the preparation with mineral oil. However, such recordings can only be made in acute experiments in anaesthetized or curarized animals and such conditions are not ideal for studying centrally acting drugs whose effects may be modified by anaesthesia. Schmitt, Schmitt & Fenard (1974) described implantation of electrodes in the dog and we have adopted this technique for chronic recordings in the rabbit.

Rabbits of 2-3 kg were anaesthetized with halothane and the abdomen opened transversely along the right costal margin. The right greater splanchnic nerve was located anterior to the crus of the diaphragm and a 1.5 cm length cleared of perinephnic fat. The nerve was then transfixed by the two prongs of a bipolar electrode. The electrode tips consisted of 0.3 mm stainless steel wire which had been sharpened electrolytically and mounted in a block of epoxy resin 3 mm apart. Flexible wires from the electrode were led out onto the skin of the back. During implantation electrical activity was monitored via a low level D.C. amplifier and oscilloscope (Tektronix USA) and when a satisfactory signal was obtained the electrode was fixed in position by the application of acrylic dental cement. Several grams of this material were deposited over the nerve and electrode. The abdomen was then closed and an indifferent electrode stitched beneath the skin.

Recordings have been made in the conscious animal up to 30 days after implantation. The pattern of electrical activity is characteristically phasic, bursts of activity being synchronized with

respiration. The discharge amplitude varies between 20 and 100 μv. In 20 consecutive preparations, 12 animals had satisfactory recordings made on the day after implantation. If the signal was satisfactory on the first day it remained so thereafter although animals were lost when wires were bitten through. Integrated activity was recorded in 7 animals for 20 min on consecutive days following implantation. Activity was greatest on day 1 and stabilized on days 4 and 5 at about 50% of the initial value. Reduction in arterial pressure with intravenous sodium nitroprusside or haemorrhage produced reflex rises in integrated activity. Anaesthesia with sodium pentobarbitone 30 mg/kg i.v. significantly reduced splanchnic nerve activity to 58 ± 8% (s.e mean, $n = 5$) of control 5 min after injection.

Rabbits are particularly suitable animals for these experiments because they remain quiet when placed in individual boxes and thus stable recordings of sympathetic activity can be made for several hours. We have used the technique for the evaluation of central and peripheral effects of beta blocking drugs (Lewis & Haeusler, 1975) and it has potential use in monitoring sympathetic nervous activity in chronic experiments.

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In vitro methods of assessing the physiological activation of macrophages in vivo

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The activation of macrophages, as assessed by a spreading and vacuolation of the cytoplasm and increases in respiratory and lysosomal enzyme activity, has been shown to occur during infection and various chronic inflammatory reactions. There is much circumstantial evidence that this activation *in vivo* is mediated by lymphokine.

In vitro, macrophages can be activated by prolonged contact with lymphokine (Nathan, Karnovsky & David, 1971; Nathan, Remold & David, 1973; Nath, Poulter & Turk, 1973). This activation only occurs however after the initial effects of lymphokine (which result in migration inhibition) have waned. The initial changes to the physiology of the macrophage appear to be an alteration in the utilization of the hydrogen liberated by the hexose monophosphate shunt, which results in reduced biosynthetic potential and a decrease in cellular permeability. These changes occur concurently with an inhibition of migrating ability as seen in the capillary tube assay. After 48-72 h of contact with lymphokine, these initial effects are reversed and increases in hexose monophosphate shunt activity and biosynthesis are seen as well as morphological changes, which result in the macrophages appearing similar to cells activated *in vivo*.

Because of this biphasic effect, it was felt that any contact with lymphokine *in vivo* would result in an altered response to subsequent lymphokine contact *in vitro*. This hypothesis has been tested by removing macrophages from animals at various times during a chronic protozoal infection (Leishmaniasis) and also after reinfecting immune animals and then recording the subsequent response of these cells to the lymphokine *in vitro*.

This was done by assaying the ability of these macrophages to respond in direct and indirect migration inhibition assays, and also by examining the effect of lymphokine contact on the activity of the hexose monophosphate shunt as detected by cytochemical tests for glucose-6-phosphate dehydrogenase activity.

The results of these studies indicate that changes in the status of macrophages during infection and following attempted reinfection can be detected by these methods, and these changes are consistent with the hypothesis that macrophage activation *in vivo* is mediated by contact with lymphokine.

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