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Department of Medicine, University of Manitoba, and The Manitoba Institute of Cell Biology, Winnipeg, Manitoba R3E 0V9, Canada

BINKOH YODA
BRENT A. SCHACTER
LYONEL G. ISRAELS

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Interaction of tricyclic antidepressants with opiate receptors

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Tricyclic antidepressants have been reported to have an analgesic effect [1,2] and some of them have been successfully used to alleviate chronic pain in man [3–6]. It is not, however, clear whether the analgetic effect of antidepressants exists in animals, due to the fact that different methods were used to measure pain [7,8], nor is the mechanism through which antidepressants exert their analgesic effects known [7]. We wish to report evidence for a direct

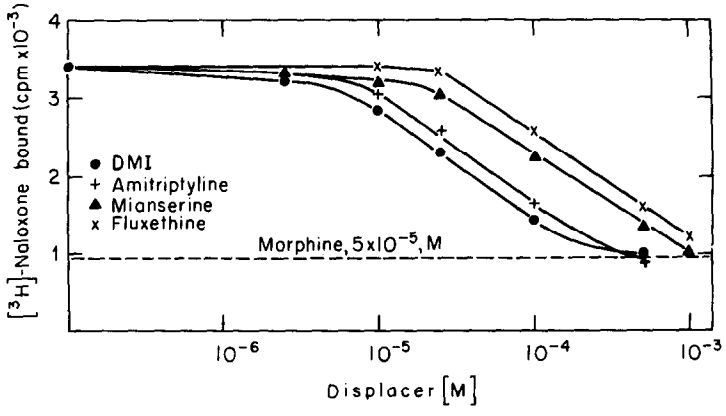

Fig. 1. Displacement of ^{3}H-naloxone binding by antidepressants. Female Wistar rats (3 months old) from the breeding colony of the Hormone Research Department of the Weizmann Institute of Science, were decapitated, their forebrains quickly removed, weighed and homogenized in 50 mM Tris–HCl Buffer, pH 7.7, in a polytron homogenizer. The homogenate was spun at 30,000 *g* for 10 min, the supernatant decanted and the pellet resuspended in buffer, homogenized and incubated for 45 min at 37°. Following a second centrifugation at 30,000 for 10 min, the pellet was resuspended in buffer and homogenized. A final volume of 200 ml per 3 of the original, weighed forebrains was used. Tissue suspension (2 ml) was added to test tubes containing 5 nM ^{3}H-naloxone (N.E.N. specific activity 15.2 Ci/mmole, 1 mCi/ml) together with (a) an unlabelled inhibitor (10^{-5}M naloxone or 5×10^{-5}M morphine); or (b) the various antidepressants or buffer to a final volume of 2.3 ml. After incubating for 45 min at room temperature (25°) the contents of each test tube were filtered through GF/B filters and washed 4 times in 5 ml ice cold buffer. The filter papers were shaken with a toluene triton scintillation mixture in vials and counted in a Packard Tri-Carb Scintillation Counter.

interaction of tricyclic antidepressants with opiate receptors, which could account for their analgesic properties in both man and animals and also, perhaps, partially account for their antidepressive action.

Recently, we have reported the specific binding of the tricyclic antidepressant ^{3}H-desipramine (DMI) to brain tissue [9]. This binding was shown to be multireceptorial. Moreover, the opiate drugs naloxone and morphine were found to be capable of a small but consistent (about 15–30 per cent) displacement of the specific binding of ^{3}H-DMI. This suggests that opiate receptors may be a target of antidepressant action. To examine this possibility, we have studied the ability of antidepressants to displace the specific binding of ^{3}H-naloxone in a crude membrane preparation from rat brain (Fig. 1).

All of the antidepressants tested were capable of a complete displacement of the specific binding of naloxone (defined as the difference between total binding and the binding in the presence of 10^{-5}M cold naloxone or 5×10^{-5}M morphine). All the tricyclic drugs have very similar IC_{50} values, between 21 and 34 μM (Table 1). The non-tricyclic drugs, mianserine and fluoxetine, have lower affinity. We have shown that upon injection of 10–15 mg/kg DMI, the levels in the brain reach values equivalent to 20–40 μM [10]. Thus, although of low affinity, the interaction of antidepressants with opiate receptors is likely to occur *in vivo*. Interestingly, similar findings were reported for neuroleptic drugs, e.g. CPZ [11] which are structurally related to tricyclic antidepressants.

In order to support these *in vitro* experiments, we have tested DMI and amitriptyline for their analgesic action in the rat. Adult female rats received increasing shock intensities until they began to jump and vocalize. On injecting either DMI (10 and 15 mg/kg, i.p.) or amitriptyline in similar doses, the pain threshold rose in proportion to the dose. The analgetic effect of these tricyclics was completely antagonized by an i.p. injection of naloxone, 2 mg/kg (Fig. 2). It has been suggested that antidepressants act by the inhibition of monoamine re uptake [12]. It has also been reported recently that tricyclics interact with monoamine [13,14], acetylcholine [15] and histamine [16] receptors, though the involvement of these receptor interactions in the clinical efficacy of antidepressants is still obscure. However, both endorphins and enkephalins have been suggested recently to be involved in the etiology of mental illness [17]. It may be, therefore, that the interaction of antidepressants with the opiate receptors accounts not only for their analgesic effect, but also for part of their antidepressive action.

Table 1. IC_{50} values of antidepressants towards the specific binding of 3H-naloxone, 5nM. Each value is the mean ± S.D. of 3–4 determinations.

Compound	$IC_{50}(\mu M)$
DMI	25 ± 2.5
Amitriptyline	33 ± 3.5
Mianserine	120 ± 10
Fluoxetine	165 ± 25
Nortriptyline	34 ± 0.5
Imipramine	21 ± 3.0
Doxepin	33 ± 6.0
Dibenzepine	32 ± 5.0

Isotope Department,
Weizmann Institute of Science,
Rehovot,
Israel

ANAT BIEGON
DAVID SAMUEL

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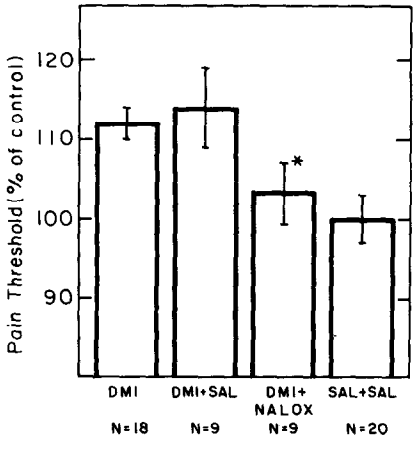

Fig. 2. The reversal of antidepressant-induced analgesia by naloxone. Adult female rats were placed in a transparent plastic cage with a grid floor, connected to a shock generator and scrambler. Footshock was delivered in 3 × 3 sec pulses in 30 sec at increasing intensities. The intensity was increased from 0.2 to 3.0 mA, until the animal jumped and vocalized. This procedure was repeated twice in order to establish the basal threshold of pain. The tricyclic antidepressant DMI (10 mg/kg), was injected, i.p. and the rats tested 20 min later. They were then injected with either saline or naloxone, 2 mg/kg i.p. and retested after 20 min.* Different from other DMI groups, $P < 0.05$, Student's *t*-test.

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Characterization of blood disappearance and tissue distribution of [^{3}H]cannabidiol

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Although cannabidiol (CBD), which is generally present in cannabis, does not possess the psychoactivity of Δ^9-tetrahydrocannabinol (THC), it is not devoid of pharmacological activity. For example, CBD can inhibit hepatic drug metabolism [1–4] and also possesses anticonvulsant properties [5, 6]. In view of the common use of marihuana, it is important to establish the time course during which an acute dose of CBD may be expected to exert its effects. Since the pharmacokinetics of CBD have not been examined, these studies have evaluated the kinetics of cannabidiol-1′,2′-[^{3}H] ([^{3}H]-CBD) disappearance from the blood of rats following intravenous (i.v.) and intragastric (i.g.) administration. The i.v. route of injection permitted an assessment of the rate of drug distribution, an observation not directly afforded by i.g. treatment. Since our previous studies [7] had demonstrated that tritiated water was an apparent product of the metabolism of Δ^9-tetrahydrocannabinol-1′,2′-[^{3}H] ([^{3}H]THC), which could modify significantly the estimate of the rate of disappearance of THC metabolites, these experiments also evaluated the possibility that tritiated water was produced from [^{3}H]CBD.

Furthermore, in a previous study [3] we observed that pentobarbital-induced sleeping time was prolonged significantly in rats at 21.5, 40 and 63 hr following an acute dose of CBD (23.4 mg/kg) administered i.g. in a marihuana extract. Our experiments suggested that this effect resulted from an inhibition of hepatic pentobarbital metabolism by CBD. This conclusion was consistent with reports by Paton and Pertwee [1] and Fernandes *et al.* [2] that CBD inhibited the metabolism of phenazone and hexobarbital, respectively. However, a direct relationship between the long-lasting inhibition of pentobarbital metabolism and a prolonged presence of unchanged CBD and/or its metabolites in the liver had not been established. We determined [3] that the CBD-induced prolongation of sleep was not due to an increase in the sensitivity of the brain to pentobarbital. In fact, animals which had received CBD, 21.5 hr before pentobarbital injection, recovered the righting reflex at higher blood pentobarbital concentrations compared to vehicle-treated controls, suggesting that CBD decreased the sensitivity of the brain to pentobarbital. Whether the latter effect was associated with the brain levels of CBD and/or its metabolites had also not been explored. Accordingly, the experiments described below determined the blood and organ concentrations of [^{3}H]CBD and its metabolites at the time points studies previously [3].

Male Wistar rats, 215–235 g, were housed individually in stainless steel hanging cages, with Teklad pellets and water available *ad lib.* throughout all experiments. [^{3}H]CBD and unlabeled CBD, each having a purity > 98 per cent, were supplied by NIDA through the courtesy of Dr. M. C. Braude.

The first experiment, initiated at 9:00 a.m., was carried out to characterize the disappearance of [^{3}H]CBD and total ^{3}H from the blood during the first 24 hr following drug administration. In one group of rats (N = 8), [^{3}H]CBD sp. act. 3.1 μCi/mg) was administered i.g. in olive oil solution (4 ml/kg) at a dose of 23.4 mg/kg. This dose had been used in the earlier CBD/pentobarbital interaction study [3]. Another group (N = 8) received [^{3}H]CBD (sp. act. 7.5 μCi/mg) in a 2% Tween-80–saline suspension (1 ml/kg) at a dose of 4 mg/kg as an i.v. bolus injection [3]. The 4 mg/kg dose of CBD was selected to be consistent with other cannabinoid interaction studies to be published elsewhere.

Tail tip blood samples were collected serially with heparinized micropipets beginning at 1 hr following i.g. administration, and at 1 min after i.v. injection of [^{3}H]CBD, and continuing for 24 hr. Two 50 μl blood samples were transferred to paper Combusto-Cones (Packard Instrument Co., Downers Grove, IL) for oxidation in a Packard, model 306, Sample Oxidizer in preparation for liquid scintillation counting (Searle Analytic, Isocap 300). One of these blood samples was oxidized immediately, but the other was air dried before analysis. The third blood sample (100 μl) was immediately mixed with 100 μl of sodium citrate solution (3.8%,w/v) and was extracted with 2 ml *n*-heptane to remove unchanged [^{3}H]CBD for liquid scintillation spectrometry, as described previously [8]. In pilot experiments, thin-layer chromatography and gas chromatography-mass spectrometry (Finnigan, 1015D, 6100 MS Data System) confirmed that > 95 per cent of the radioactivity present in *n*-heptane extracts of blood collected from rats 1–6 hr after i.v. [^{3}H]CBD treatment was unchanged [^{3}H]CBD; that is, *n*-heptane extracted only trace amounts of [^{3}H]CBD metabolites. Also in preliminary experiments, 97–99 per cent of [^{3}H]CBD added to blood was extracted by the procedure. Therefore, all [^{3}H]CBD concentration data were corrected to 100 per cent, using a 98 per cent recovery factor.

The second experiment was designed to evaluate the disappearance of [^{3}H]CBD and total ^{3}H from the blood, liver and brain from 21.5 to 84 hr after [^{3}H]CBD (23.4 mg/kg, i.g.) administration, a time period which coincided with that studied previously [3]. The time of drug treatment was selected so that all animals were killed between 9:00 and 10:00 a.m. Upon decapitation at 21.5, 40, 63 and 84 hr (N = 8/time), three neck blood samples were collected and analyzed, as described above. The brain and liver were removed immediately and placed on ice. Two small segments (approximately 100–150 mg) were dissected at random sites from each organ. Fresh weights were taken, and one segment of each organ was oxidized immediately. The other was lyophilized before analysis for ^{3}H.

The remainder of the fresh brain and approximately 1 g of each liver were assayed for unchanged [^{3}H]CBD. The tissues were homogenized in 2 vol. of 0.10 M phosphate buffer, pH 7.0, and were extracted twice with 3 ml of *n*-heptane each time. The extracts were assayed for ^{3}H [8]. Pilot experiments showed that > 90 per cent of the radioactivity present in *n*-heptane extracts of organs of rats that had been treated i.g. with [^{3}H]CBD, 24 hr earlier, was unchanged [^{3}H]CBD. Furthermore, the procedure