vision mirror in a sound attenuated chamber measuring 82 cm^2 by 70 cm high. Daily injections of THC (0.1 mg/kg to 3 rabbits) or CBN (15 mg/kg to 3 rabbits) were continued until no convulsions occurred. The following, and subsequent days, if necessary, the dose of cannabinoid was increased until a convulsion was again elicited. This higher dose was given daily until the convulsions ceased. On the next day, the animal received an injection of the cannabinoid (0.1 mg/kg THC or 15 mg/kg CBN) that had not been previously administered. 1 week later the original dose of cannabinoid was given. If a convulsion was not elicited, a higher dose was injected.

Results. As shown in the table, A, although there was some individual variation, all rabbits exhibited tolerance to the convulsant effects of THC. The tolerance was first exhibited following repeated administration of 0.1 mg/kg THC. An increase in dose to 0.5 mg/kg was sufficient to reinstate the convulsive behaviors and tolerance developed after daily administration of this dose. On the day following occurrence of tolerance to the higher dose of THC, a dose of CBN (15 mg/kg), shown previously to reliably elicit convulsions in our rabbit population[14], was given. In all 3 rabbits, no convulsions were precipitated, indicating that cross-tolerance occurred from THC to CBN. 1 week after CBN administration each subject was injected with 0.1 mg/kg THC. In 2 out of 3 animals a convulsion occurred, indicating that tolerance had been lost. The 3rd rabbit required 0.5 mg/kg prior to exhibiting a loss of tolerance. The table, B, illustrates the results of repeated dosing with CBN. Tolerance occurred to the 15 mg/kg dose of CBN and this could be reversed by higher doses of CBN (20 or 30 mg/kg). After daily administration, tolerance occurred to the higher CBN dose. Subsequent injection of a convulsive dose of THC (0.1 mg/kg) did not evoke a convulsion, indicating a cross-tolerance from CBN to THC. CBN (15 mg/kg) again elicited convulsions when administered after a 1-week drug-free interval.

Discussion. In the THC-SS rabbits, tolerance developed to the behavioral convulsant effects of both THC and CBN. This was evidenced by both an abolition of response after repeated administration of the same dose and a reinstitution of convulsions subsequent to a higher dose of each cannabinoid. Tolerance to convulsions was not permanent since each cannabinoid elicited convulsions in the previously tolerant THC-SS rabbits after a 1-week drug-free interval. Reversible tolerance to the rabbit convulsions with THC reaffirms previous findings[14], and is congruent with the development of tolerance to THC effects across species[3-6]. The present findings of tolerance to CBN-induced convulsions and of symmetrical cross-tolerance between CBN and THC are novel. These data suggest a similar mechanism of action of the 2 cannabinoids which is compatible with previously reported structure-activity considerations of cannabinoids[2]. Moreover, these data support findings that CBN is capable of producing psychoactive effects in humans that are qualitatively similar to those elicited by THC. 2 human studies[9,10] have demonstrated that both CBN and THC, given by i.v. infusion, produced a psychological 'high' and tachycardia. The dose required for CBN was about 10-fold higher than that for THC. However, the results of 2 other studies indicate that humans receiving up to 400 mg/kg of oral CBN did not experience any of the mental and physical effects characteristic of an oral 10 mg dose of THC[7,8]. The discrepancies between the findings of these human studies are difficult to explain since many relevant details are absent from the reports. Nevertheless, numerous studies indicate that CBN is a less potent THC-type compound[9-13]. This suggests that a relatively high concentration of CBN in marijuana could conceivably contribute to the pharmacological effects of the plant material.

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Electro-osmotic and iontophoretic release of noradrenaline from micropipettes[1]

P. Bevan[2], C.M. Bradshaw, R.Y.K. Pun, N.T. Slater and E. Szabadi

Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT (England), 14 February 1980

Summary. The relative contributions of electro-osmosis and iontophoresis to the electrophoretic release of noradrenaline from micropipettes were examined. Electro-osmosis was responsible for 23.1% (±5.0%) of the total rate of release.

The technique of microelectrophoresis is used to apply minute quantities of drugs into the immediate vicinity of single neurones. Drugs are released from ionized solutions contained within glass micropipettes by the passage of currents of appropriate direction through the solutions. In most studies of central neurones, multi-barrelled micropipettes are used, consisting of several capillary tubes converging into a common tip[3]. The electrophoretic release of drugs from micropipettes involves 2 physico-chemical processes: a) the ejection of ionized drug molecules by iontophoresis, and b) the efflux of small volumes of the drug solution due to electro-osmosis. Of the two, iontophoresis is assumed to make the greater contribution to total drug release, at least in the case of well-ionized drugs[3]. We have attempted to assess the contribution of electro-osmosis to the release of noradrenaline (NA) by measuring the rate

at which a practically unionized molecule, glucose, is released concomitantly with NA during the passage of electrophoretic currents.

Method. Ten 6-barrelled micropipettes were prepared as described previously[4]; 3 barrels (1–3) of each were filled with a mixture of [^{14}C]-NA bitartrate (0.05 M; 1 mCi/mmole) and glucose (0.0167 M), and the remaining 3 (4–6) with a mixture of NA bitartrate (0.05 M) and [^{14}C]-glucose (0.0167 M; 1 mCi/mmole). In each micropipette the rate of efflux of radioactive material was measured during a series of 10-min periods. In calculating the rate of electrophoretic release of [^{14}C]-NA or [^{14}C]-glucose, the mean rate of spontaneous release of radioactive material was subtracted from the total rate of release of radioactive material. The rate of release of [^{14}C]-NA was measured during the passage of +25, +50, +75 and +100 nA through barrels 1–3 (4 samples at each current), and the rate of release of [^{14}C]-glucose was measured during the passage of identical currents through barrels 4–6. The latter measurements were used to calculate the rate of efflux of the solution, which in turn was used to calculate the rate of release of NA by electro-osmosis (R_e). By subtracting R_e from the total rate of electrophoretic release of NA (R_t), the rate of iontophoretic release of NA (R_i) was calculated. 'Apparent' and 'real' transport numbers for NA (n_a and n_r) were calculated by substitution in Faraday's equation:

$$n_a = R_t \cdot z \cdot F/3i \text{ and } n_r = R_i \cdot z \cdot F/3i$$

(R_t and R_i are in moles · sec^{-1}; z is the valency of NA = 1; F is Faraday's constant; i is the current in A passed through each of the 3 barrels). An additional experiment was carried out using 4 micropipettes in order to determine whether the addition of glucose to the NA solution distorted the electrophoretic release of NA. 3 barrels were filled with [^{14}C]-NA bitartrate (0.05 M; 1 mCi/mmole), and the remaining 3 with a mixture of [^{14}C]-NA bitartrate (0.05 M; 1 mCi/mmole) and glucose (0.0167 M). The rate of release of NA from the barrels containing these 2 solutions was measured as described above.

The specific conductivity of the NA bitartrate solution was measured at 25 °C using a Wayne Kerr Universal Bridge B224.

Results. The rate of fluid efflux was linearly related to current intensity; figure 1 shows the results obtained from 10 micropipettes. The mean rate of efflux was 0.815 pl · barrel^{-1} · min^{-1} · nA^{-1}. R_t, R_i and R_e were all linearly related to ejecting current intensity (figure 2); the mean 'apparent' transport number of NA was 0.287 (±0.022), and the mean 'real' transport number was 0.220 (±0.025). Electro-osmosis accounted for 23.1% (±5.0%) of the total electrophoretic release of NA.

The presence of glucose did not significantly influence the rate of release of NA (t-test, $p > 0.1$), the mean apparent transport number obtained from the 4 pipettes used in the 'additional' experiment (see above) being 0.223 (±0.009) and 0.226 (±0.019) in the absence and presence of glucose, respectively.

The specific conductivity of the 0.05 M NA bitartrate solution was 1.86 mmho · cm^{-1}.

Discussion. The relative contribution of electro-osmosis to the total release of NA (23.1%) is considerably greater than the 11% estimated for acetylcholine released from a 3 M solution[5]. This probably reflects the more dilute solution used by us. The electrophoretic release of drugs from micropipettes is analogous to the flow of current through 3 parallel resistors, the fractions of the current flowing through the resistors corresponding to the proportions of the electrophoretic current carried by iontophoretic transport of drug ions, iontophoretic transport of foreign ions, and electro-osmotic outflow. The use of relatively dilute solutions reduces the conductivity and increases the electroosmotic mobility of the solution (for discussion, see Krnjević et al.[5], and thus favours the passage of current by electro-osmosis. The addition of foreign ions (as, for instance, when the pH of a drug solution is adjusted with NaOH, or when NaCl is added to solutions of alkaloids in order to facilitate the passage of ejecting currents) reduces the transport number of the drug ion and increases the conductivity of the solution, thereby reducing both iontophoretic and electro-osmotic drug release[6].

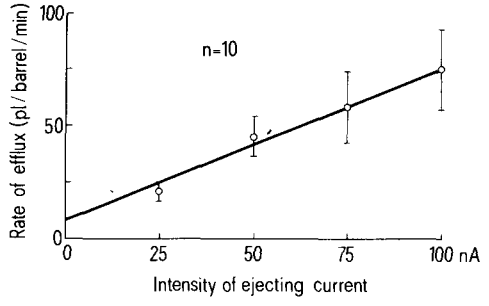

Fig. 1. Rate of electro-osmotic efflux of solution from 10 6-barrelled micropipettes (± SEM), as a function of intensity of ejecting current passed through each barrel. The line was fitted by the least squares method.

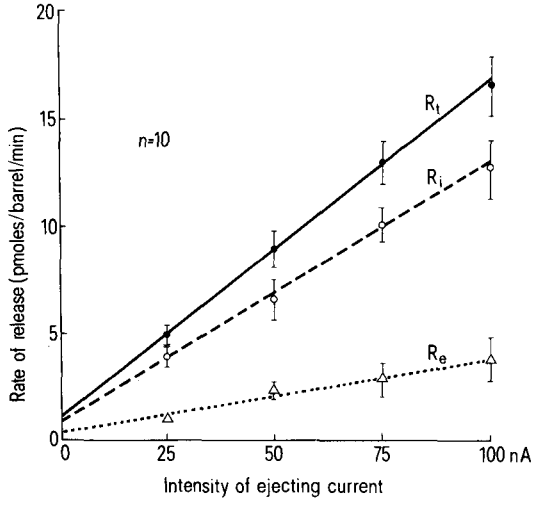

Fig. 2. Rate of release of noradrenaline from 10 6-barrelled micropipettes (± SEM), as a function of intensity of ejecting current passed through each barrel. R_t, total rate of release; R_i, rate of iontophoretic release; R_e, rate of electro-osmotic release. The lines were fitted by the method of least squares.

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2 Present address: Preclinical Research, Sandoz AG, CH-4002 Basel, Switzerland.

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