Estimation and disposition of [^{3}H]benzoylecgonine and pharmacological activity of some cocaine metabolites

Benzoylecgonine (pKa 8·65, 8·80, 11·80) is known to be the major metabolite of cocaine (ref. cit. Misra, Nayak & others, 1974a). No information exists on the physiological disposition and metabolism of benzoylecgonine. Its amphoteric nature and unfavourable partition characteristics have hampered the development of a sensitive method for its estimation in biological materials. Previous methods (Misra, Pontani & Mulé, 1973a; Valanju, Baden & others, 1973; Koontz, Besemer & others, 1973; Bastos, Jukofsky & Mulé, 1974) lack adequate sensitivity for its estimation in sub-microgram range in tissues. This communication describes an improved method of assaying [^{3}H]benzoylecgonine in biological materials, its distribution and metabolism in the rat and its gross pharmacological activity in the rat and that of some other metabolites of cocaine (benzoylnorecogonine, ecgonine, norcocaine and ecgonine methylester) after intravenous and intracisternal injections.

[^{3}H]Benzoylecgonine (sp. act. 31 μCi mg^{-1}) was prepared by the hydrolysis of carrier-diluted [^{3}H]cocaine (Nayak, Misra & others, 1974) by a micro-scale adaptation of the procedure of Findlay (1954). Benzoylecgonine, ecgonine methyl ester were prepared according to Findlay (1954), benzoylnorecgonine and norcocaine by the method of Schmidt & Werner (1962) and ecgonine by the method of Bell & Archer (1960).

In vitro *recoveries of* [^{3}*H*]*benzoylecgonine from biological materials.* Two ml aliquots of diluted urine, plasma (1:5) or tissue homogenates of rats (20% in 0·9% saline) containing known concentrations of [^{3}H]benzoylecgonine in the 5–1000 ng range were transferred to 40 ml centrifuge tubes containing 1 ml non-radioactive benzoylecgonine (500 μg ml^{-1}) as carrier. To this solution 2 g solid K_2CO_3 was added (pH, 11–12) followed by 15 ml chloroform–isopropanol (2:1, v/v) and the mixture shaken for 20 min. After centrifugation for 10 min, the aqueous phase was aspirated and 10 ml organic phase evaporated to dryness at 45–50° in counting vials. The residue was dissolved in 0·5 ml methanol, 10 ml toluene phosphor added and radioactivity determined in a liquid scintillation counter. Other details on counting technique, calibration curve for calculation of benzoylecgonine concentrations in tissues and fluids have been described by Misra & Mulé (1972), Misra, Mulé & others (1973b) and Misra, Pontani & Mulé (1974b). *In vitro* recoveries of [^{3}H]benzoylecgonine from aqueous solutions in the concentration range 5–1000 ng were: 95·4 $\pm$ 0·3% (s.e.); from urine and plasma 91·0 $\pm$ 1·7% (s.e.); brain 90·0 $\pm$ 0·6% (s.e.); and liver 81·3 $\pm$ 0·9% (s.e.). Extractions at lower pH with or without saturation of aqueous phase with sodium chloride or washing of organic phase with dilute 4% K_2HPO_4 buffer lowered the recoveries. Ecgonine was also quantitatively extracted by this procedure. For the specific extraction of cocaine from biological materials see Misra & others (1974a).

Distribution of [^{3}*H*]*benzoylecgonine in the rat.* Male Wistar rats (130–160 g) were injected in the tail vein with a 10 mg kg^{-1} dose of [^{3}H]benzoylecgonine (sp. act. 6·2 μCi mg^{-1}). At chosen times, animals were lightly anaesthetized with ether and blood drawn into heparinized vacutainer tubes by cardiac puncture. Plasma was obtained immediately by centrifugation. Known weights of brain were homogenized in 0·9% saline to give a 20% homogenate and 2 ml aliquots were taken in duplicate and analysed. Plasma, diluted, was similarly treated.

For intracisternal injections, rats were lightly anaesthetized with ether and injected with [^{3}H]benzoylecgonine 1 mg kg^{-1} in 10 μl. Brains were removed 0·5 h later and analysed for benzoylecgonine. Cocaine and other metabolites were similarly injected intracisternally in volumes of 10 μl, the pH of solutions being adjusted to 6·8–7·0 with 0·1 N NaOH.

Metabolic studies. Male Wistar rats were injected intraperitoneally with 200 mg kg^{-1} benzoylecgonine and the urine was collected for 48 h, pooled, evaporated under vacuum, and the residue repeatedly extracted with methanol. The methanol-soluble portion, on preparative thin-layer chromatography (Gelman instant thin-layer chromatography media [ITLC] silica gel, 20 × 20 cm sheets) with solvent system: n-butanol–acetic acid–water (35:3:10, v/v), provided 2 major bands R_F, 0·15–0·50 (A) and 0·8–1·0 (B). These were eluted with methanol and separately rechromatographed on the same medium with ethyl acetate–methanol–conc. ammonia (15:4:1, v/v). This procedure resolved A into two compounds R_F 0·05 (minor) and 0·24 (major) and B into R_F 0·3 (minor) and 0·8 (major), respectively.

[^{3}H]Benzoylecgonine concentrations in rat brain and plasma after a 10 mg kg, i.v. injection are in Table 1. Mean peak concentrations in brain were attained 0·5 h after injection and declined to 20–28 ng g^{-1} or ml by 6 h. The ratio of mean peak concentration in brain to plasma was 0·12 (apparent partition coefficient of [^{3}H]benzoylecgonine in 1-octanol–phosphate buffer pH 7·4, 0·15 ± 0·01 s.e.). The brain to plasma ratio with [^{3}H]ecgonine (Misra, Vadlamani & others, 1974c) after a similar intravenous dose was 0·03.

Cocaine (apparent partition coefficient in 1-octanol–buffer pH 7·4, 7·6 ± 0·1 s.e.), however provided a brain to plasma ratio of 11·9, 15 min after injection of a subconvulsive 8 mg kg^{-1} intravenous dose (Misra & others, 1974a) and the peak concentrations of [^{3}H] cocaine in brain and plasma at this time were 7269 ± 177 ng g^{-1} and 612 ± 62 ng ml^{-1}, respectively. The half-lives of benzoylecgonine, ecgonine and cocaine in rat brain were 1·3, 7·8 and 0·4 h, respectively, that in plasma 0·8, 3·8 and 0·3 h, respectively.

After intracisternal injection of [^{3}H]benzoylecgonine (1 mg kg^{-1}) to the rats, approximately 5–11% of the dose g^{-1} of brain was observed 0·5 h post-injection. Extraction of rat brains and thin-layer chromatography of the extract provided no evidence for *N*-dealkylation of benzoylecgonine to benzoylnorecgonine.

The compounds of R_F 0·24 and 0·8 were shown to be ecgonine and benzoylecgonine by co-chromatography. A minor compound R_F 0·3 showed the presence of a phenolic group by Folin-Ciocalteau and $FeCl_3$-$K_3Fe(CN)_6$ reagents. A compound of R_F 0·05 gave a positive test for glucuronide (Dische, 1947) and on hydrolysis with β-glucuronidase at pH 6·8 generated the phenolic compound. It was also intensely positive to cobalt nitrate reagent (Azouz, Parke & Williams, 1953). These experiments provided evidence for the existence of benzoylecgonine and the formation of ecgonine as a major metabolite and two minor metabolites, i.e. a phenolic compound (*p*-aromatic hydroxylation) and its conjugate as glucuronide.

Table 1. *Distribution[a] of [^{3}H]benzoylecgonine in brain and plasma of male Wistar rats after a 10 mg kg^{-1} dose by intravenous injection.*

	0·25 h	0·5 h	1 h	3 h	6 h	Half-life (h)
Brain	208 ± 14	366 ± 114	283 ± 52	111 ± 7	20 ± 2	1·3
Plasma	4150 ± 282	3088 ± 175	2008 ± 284	338 ± 115	28 ± 4	0·8
Brain/plasma ratio	0·05	0·12	0·14	0·32	0·71	

(a) Data are mean values ± s.e.m. (ng g^{-1} wet tissue weight or ml fluid) of 6 determinations from 3 animals at each time.

No observable pharmacological effects were noted with ecgonine and ecgonine methyl ester after high doses (200 mg kg^{-1}, i.v. or 10 mg kg^{-1}, i.c.) and also with benzoylecgonine after doses of 250 mg kg^{-1} (i.v.). Cocaine and norcocaine, the lipophilic compounds, were active by both intravenous and cisternal routes. A 20 mg kg^{-1} (i.v.) injection or 1–2 mg kg^{-1} (i.c.) injection of cocaine and norcocaine to the rats caused excessively rapid heart beat, convulsions and death within 3–5 min. Lower doses (5–10 mg kg^{-1}, i.v. or 0·5–1·0 mg kg^{-1}, i.c.) produced similar results without mortality, and the effects lasted for 5–20 min. At 1 mg kg^{-1} (i.c.) benzoylecgonine, piloerection, running and jumping activity, jerking, rapid breathing and squeaking appeared within 5 min and these effects lasted for approximately 4 h; with higher doses (2 mg kg^{-1}) these effects became more violent without ensuing mortality. Benzoylnorecgonine (100 μg kg^{-1}, i.c.) produced effects similar to benzoylecgonine within 1 min of administration and death within 30 min. The potent stimulant effects observed with these two compounds on intracisternal injections were dose-dependent and their nature were distinctly different from those observed after the injections of cocaine or norcocaine.

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