Br. J. Pharmac. (1978), **63**, 3–6.

EFFECTS OF PROPYLBENZILYLCHOLINE MUSTARD ON INJECTION INTO THE LIQUOR SPACE OF CATS

F. BERGMANN[1] & W. FELDBERG

National Institute for Medical Research, Mill Hill, London NW7 1AA

In unanaesthetized cats the effects were examined of propylbenzilylcholine mustard (PrBCM) on injection into the cannulated cerebral ventricles and cisterna magna. Extreme motor excitation, vocalization, shivering leading to fever, tachypnoea, panting, piloerection and salivation were produced on ventricular, vigorous scratching bouts on cisternal, injections. The sites of these actions are discussed. None of the effects was produced by atropine similarly injected. All effects were suppressed by anaesthetizing doses of pentobarbitone sodium injected intraperitoneally.

Introduction Propylbenzilylcholine mustard (PrBCM) was found to be a potent irreversible ligand for muscarinic receptors in longitudinal muscle strips of guinea-pig small intestine (Burne, Hiley & Young, 1974a) and in homogenates prepared from cerebral cortices of mice, rats, guinea-pigs, pigs, dogs and monkeys (Burgen, Hiley & Young, 1974b). In rats it was shown that PrBCM (80 μg) was inactive when injected into a carotid artery but produced convulsions when injected into a lateral cerebral ventricle (Bergmann, Birdsall, Burgen & Hulme, unpublished observations).

In the present experiments, PrBCM was injected into the cerebral ventricles and into the cisterna magna of unanaesthetized cats to find out whether it produced effects like those produced in rats, and if so, whether they could be localized to special parts of the brain and would resemble those produced by atropine, similarly injected.

Methods The experiments were done on cats of either sex weighing between 2.1 and 3.6 kilograms. In an aseptic operation under pentobarbitone sodium anaesthesia, a Collison cannula was implanted either into the left lateral or the anterior part of the third ventricle as described by Feldberg & Shaligram (1972), or just above the cisterna magna as described by Feldberg, Gupta, Milton & Wendlandt (1973). After recovery from the operation the injections of the drugs were made without anaesthesia in a volume of 10–50 μl. For the injections into the cisterna magna the atlanto-occipital membrane was first pierced with a hollow needle inserted through the shaft of the cannula as described by Feldberg *et al.* (1973) and the injection of the drug solution was followed by an injection of 0.1 ml 0.9% w/v NaCl solution (saline).

In several experiments rectal temperature was recorded at room temperature, 20–23°C, with a thermistor probe (Yellow Spring Instrument Co.) inserted about 10 cm deep into the rectum and held in position by adhesive tape attached to the protruding end of the probe and gently wrapped around the base of the tail. Temperature was monitored continuously by a Kent multichannel recorder and Figure 1 reproduced in this paper is plotted directly from the tracing obtained in this way.

Drugs used The PrBCM was synthesized for us by Dr N. Birdsall according to the method of Young, Hiley & Burgen (1972). From a concentrated stock solution in ethanol appropriate dilutions were made with saline immediately before each experiment. The amounts of atropine sulphate (BDH Ltd) given in the text refer to the salt.

Results

Injections into the cerebral ventricles The effects of PrBCM injected into a lateral and into the third ventricle were similar except that the injections into the third ventricle were more potent; a dose of 8 μg injected into the third and a dose of 20 μg injected into a lateral ventricle, produced about the same effect. Following these injections, shivering began within a minute or two, and quickly became vigorous and widespread. The next effect was vocalization. It began with periods of miaowing which became more frequent and of longer duration. Gradually the miaowing changed to growling and yelping. Later,

[1] Permanent address: Department of Pharmacology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

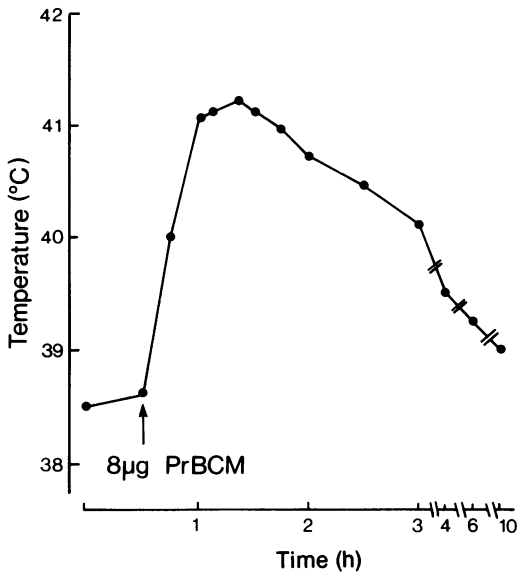

Figure 1 Rectal temperature obtained from an unanaesthetized cat weighing 3 kg; at the arrow, 8 μg of propylbenzilylcholine mustard (PrBCM) was injected into the cannulated third ventricle.

tachypnoea, panting, salivation, piloerection and ear twitching were observed; later again, periods of intense excitation alternated with periods of a more restful condition. During the periods of excitation, the cat would suddenly charge blindly ahead, or jump up to and cling onto the side or the roof of the cage, the pupils being maximally dilated. The cats showed compulsive biting; care had to be taken to prevent them biting through the lead of the rectal probe by offering them instead a pencil, on which they would clamp their teeth and eventually gnaw through. The effects were abolished by anaesthetizing the cats with intraperitoneal injection of pentobarbitone sodium. If not abolished in this way, they diminished 2–3 h after the injection, and the cats recovered fully within 24 hours.

The shivering resulted in fever and the greater potency of PrBCM in raising body temperature on injection into the third rather than into a lateral ventricle became evident when recording rectal temperature. For instance, an injection of 8μg into a lateral ventricle produced no rise in rectal temperature, or at most a rise of less than 0.5°C, whereas on injection into the third ventricle this dose of PrBCM raised rectal temperature by about 2°C within 20 minutes. On injection into a lateral ventricle, 20 μg had to be injected to produce a hyperthermia of the same order. On injection into the third ventricle even 1 μg was effective and produced a rise of nearly 1°C in 30 minutes.

An experiment in which an injection of 8 μg of PrBCM into the third ventricle caused a rise of over 2.5°C is illustrated in Figure 1. Temperature began to rise within the first minute of injection together with shivering which quickly became vigorous and widespread. Five minutes after the injection there was tachypnoea with long periods of panting. Salivation was seen about 20 min after the injection. Temperature remained at about 41°C for about 45 min and then fell, but gradually only, so that a temperature of 39°C was not reached until 10 h after the injection.

Since the hyperthermic effect of PrBCM was also obtained when it was injected about an hour after an intraperitoneal injection of indomethacin (2 mg/kg), the hyperthermia is not produced indirectly through increased synthesis of prostaglandin.

Injections of 150 μg of atropine sulphate into a lateral or into the third ventricle did not produce any of the effects obtained with PrBCM.

Injections into the cisterna magna Injections of 20–50 μg of PrBCM led initially to sedation, but after 20–30 min the cat began to scratch itself, at first every few minutes for a few seconds, but later more and more frequently, and the bouts of scratching lasted longer, up to 30 s, and became very intense, often wounding the skin at the side and front of the ears. The scratching bouts were sometimes followed by periods of vigorous wiping of the face with a forepaw and of licking the forepaws. In one cat the condition of frequent intense scratching bouts continued unabated for 2 h, but stopped when the cat was then anaesthetized by an intraperitoneal injection of nembutal. In another cat the scratching bouts assumed, after a short time, a paroxysmal character, but again ceased within a few minutes of an intraperitoneal injection of nembutal.

Defaecation and micturition were also observed after the intracisternal injections of PrBCM, but not hyperthermia.

Intracisternal injections of atropine sulphate (150–300 μg) produced sedation like PrBCM, but did not elicit scratching movements.

Discussion PrBCM was found to be a potent substance when introduced into the liquor space of unanaesthetized cats but the effects were not reproduced by atropine sulphate similarly applied. In the present experiments, the injection of 150 μg of atropine sulphate into the cerebral ventricles produced no changes in behaviour or in body temperature. In previous experiments (Feldberg & Sherwood, 1954), however, the injections of up to 300 μg atropine sulphate produced increased liveliness and restlessness, and, as

in the present experiments with PrBCM, tachypnoea, panting and profuse salivation. The increased restlessness and liveliness cannot be equated with the extreme motor excitement obtained with PrBCM, leading to blind charging ahead, jumping up to and clinging to the roof and sides of the cage and compulsive biting. These effects are also obtained with intraventricular injections of nicotine (Hall & Reit, 1966) and during the initial stage of tubocurarine action (Feldberg & Sherwood, 1954). With tubocurarine this excitement is followed after a short time by tonic-clonic convulsions which result from an excitatory action on the hippocampus, a structure readily accessible from the lateral ventricles (Feldberg & Fleischhauer, 1962; 1963). The extreme motor excitement following intraventricular injections of PrBCM on the other hand, results from an action on structures in the walls of the third ventricle, probably the hypothalamus, because from this ventricle the behavioural effect was elicited with smaller doses than from a lateral ventricle. We have not examined the effects of larger doses of PrBCM injected into a lateral ventricle to see whether they would activate the hippocampus as well and cause convulsions, as in rats.

Substances injected into the third ventricle pass rapidly into the subarachnoid space and may then act on structures of the brain stem and upper cervical cord. This mode of action may account for the observed salivation, tachypnoea, panting and ear twitching because these effects, when produced by intraventricular nicotine, were shown to occur after the nicotine had passed into the subarachnoid space. The first three effects could be attributed to an action on structures in the brain stem, the ear twitching to an action on structures in the upper cervical cord (Hall & Reit, 1966).

On the other hand, two effects of PrBCM, the vocalization and the shivering leading to fever, can be attributed to an action on structures in the walls of the third ventricle. Again, these effects were not obtained with atropine but have been observed with other drugs similarly injected. Vocalization is a characteristic effect of intraventricular tubocurarine (Feldberg & Sherwood, 1954), nicotine (Hall & Reit, 1966) and morphine (Feldberg & Shaligram, 1972) and results probably from an action on the posterior hypothalamus. Shivering and fever are characteristic effects of intraventricular tubocurarine, morphine, prostaglandins, endotoxins, and lipid A, and result from an action on the anterior hypothalamus (for references see Feldberg, 1975).

The scratching bouts evoked by intracisternal injections of PrBCM were not reproduced by atropine, but are characteristic for a variety of drugs injected in this way. They are due to an action on structures situated at the dorsal surface of the cervical cord at the level of C1 and C2 (Feldberg & Fleischhauer, 1960; Domer & Feldberg, 1960) and have been described for tubocurarine, bromophenol blue, morphine, eserine, prostigmine, dyflos and fluoro-acetate (for references see Feldberg, 1963).

Although the effects produced by intraventricular and intracisternal injections of PrBCM were abolished when the cats were anaesthetized by intraperitoneal pentobarbitone sodium, we have not examined the effect of larger doses of PrBCM to see whether they would overcome the depressant effects of the anaesthetic. If so, it would facilitate a more detailed localization of the central effects produced by the mustard.

The finding that the central effects of PrBCM are not reproduced by atropine but have been obtained with other drugs can be explained in different ways. The muscarinic receptors may not be the sole receptors with which PrBCM is binding in the brain. Or the binding to the muscarinic receptors may not be as specific *in vivo* as suggested from the *in vitro* experiments. Or PrBCM may activate the muscarinic receptors before inactivating them by irreversible binding. For instance, scratching on intracisternal injection is also observed with inhibitors of cholinesterases.

F.B. was supported by a Wellcome Research Grant.

References

BURGEN, A.S.V., HILEY, R. & YOUNG, J.M. (1974a). The binding of [^{3}H] propylbenzilylcholine mustard by longitudinal muscle strips from guinea-pig small intestine. *Br. J. Pharmac.*, **50**, 145–151.

BURGEN, A.S.V., HILEY, R. & YOUNG, J.M. (1974b) The properties of muscarinic receptors in mammalian cerebral cortex. *Br. J. Pharmac.*, **51**, 279–285.

DOMER, F.R. & FELDBERG, W. (1960). Scratching movements and facilitation of the scratch reflex produced by tubocurarine in cats. *J. Physiol.*, **153**, 35–51.

FELDBERG, W. (1963). *A Pharmacological Approach to the Brain from its Inner and Outer Surface.* London: Edward Arnold.

FELDBERG, W. (1975). Body temperature and fever: changes in our views during the last decade. The Ferrier Lecture. *Proc. R. Soc., Lond. B.*, **191**, 199–229.

FELDBERG, W. & FLEISCHHAUER, K. (1960). Scratching movements evoked by drugs applied to the upper cervical cord. *J. Physiol.*, **151**, 502–517.

FELDBERG, W. & FLEISCHHAUER, K. (1962). The site of

origin of the seizure discharge produced by tubocurarine acting from the cerebral ventricles. *J. Physiol.*, **160**, 258–283.

FELDBERG, W. & FLEISCHHAUER K. (1963). The hippocampus as the site of origin of the seizure discharge produced by tubocurarine acting from the cerebral ventricles. *J. Physiol.*, **168**, 435–442.

FELDBERG, W., GUPTA, K.P., MILTON, A.S. & WENDLANDT, SABINE (1973). Effect of pyrogen and antipyretics on prostaglandin activity in cisternal c.s.f. of an anaesthetized cat. *J. Physiol.*, **234**, 279–303.

FELDBERG, W. & SHALIGRAM, S.V. (1972). The hyperglycaemic effect of morphine. *Br. J. Pharmac.*, **46**, 602–618.

FELDBERG, W. & SHERWOOD, S.L. (1954). Injections of drugs into the lateral ventricle of the cat. *J. Physiol.*, **123**, 148–167.

HALL, G.H. & REIT, E. (1966). Analysis of some central actions of nicotine injected into the cerebral ventricles of cats. *J. Physiol.*, **185**, 400–417.

YOUNG, J.M., HILEY, R. & BURGEN, A.S.V. (1972). Homologues of benzilylcholine mustard. *J. Pharmac. Pharmac.*, **24**, 950–954.

(*Received December 19, 1977.*)