THE AFFERENT PATH OF THE MILK-EJECTION REFLEX IN THE BRAIN OF THE RABBIT

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SUMMARY

The afferent path of the milk-ejection reflex has been studied in the brain of the anaesthetized lactating rabbit. Electrical stimulation was applied between a monopolar electrode in the brain and an indifferent electrode in the scalp. The brain was transected at the mid-cerebellar level to eliminate sympathetico-adrenal activation, and intramammary pressure and arterial blood pressure were monitored to detect release of neurohypophysial hormones.

In the mid-brain, the afferent path of the reflex is compact, lying in the lateral tegmentum of each side and passing forwards to lie medio-ventral to the medial geniculate body. On entering the diencephalon, the pathway on each side bifurcates: a dorsal path passing forwards in association with the extreme rostral central grey and periventricular region, and a ventral path ascending through the subthalamus. The dorsal and ventral paths reunite in the posterior hypothalamus. Delineating the pathway further forward in the hypothalamus, using a simple stimulation technique, was not possible because at this level it intermingles with efferent fibres descending from the paraventricular nucleus to the pituitary stalk. The afferent path of the reflex is concerned with the preferential release of oxytocin from the neurohypophysis, is not a major pathway for the release of vasopressin and its neural substrate in the mid-brain is believed to be the spinothalamic system of fibres.

INTRODUCTION

Although the suckling stimulus plays an important role in milk removal in many species by evoking the release of oxytocin from the neurohypophysis, comparatively little is known of the central ascending pathways involved in this release (see Cross, 1966; Tindal, 1967). Recently, however, it was found that the afferent path of the milk-ejection reflex in the mid-brain of the guinea-pig is compact and lies bilaterally in the lateral tegmentum. As it passes forwards to enter the diencephalon the pathway on each side divides into two components which reunite in the posterior hypothalamus (Tindal, Knaggs & Turvey, 1967a, b). Because of the possibility, however remote, that this pathway in the guinea-pig was atypical and represented a special case, it was thought advisable to investigate at least one other species, and we now report our findings in the rabbit.
MATERIALS AND METHODS

Fifty-nine lactating New Zealand White rabbits, weighing 3300–5840 g., were used. The day of lactation on which individual rabbits were used varied from the 11th to the 39th day and mothers were isolated from their litters 24 hr. before the day of the experiment to allow the mammary glands to become engorged with milk. On the day of experiment, anaesthesia was induced by i.v. injection of pentobarbitone sodium B.P. dissolved in distilled water (50 mg./ml.), and polyethylene cannulae (bore 1·0 mm., Portland Plastics Ltd.) were inserted into an external jugular vein and into a femoral artery. A teat was cannulated with a metal cannula and a Y-shaped Nylon cannula (Portland Plastics Ltd.) was inserted into the trachea.

The rabbit was then fixed in a Kopf stereotaxic instrument by the method of Sawyer, Everett & Green (1954) after infiltration of pressure points with 1% lignocaine hydrochloride solution (Versicaine, May & Baker Ltd.); the scalp was reflected, a plate of bone was removed from the roof of the skull and the dura mater was incised and reflected. The brain was transected at the mid-cerebellar level to eliminate sympathethico-adrenal activation (see Tindal et al. 1967b) and respiration was maintained artificially with a pump (Miniature Ideal, C. F. Palmer Ltd.). The teat and femoral cannulae led to pressure transducers (Statham P23BC, P23AC) which were connected to two channels of a Grass Model 7 Polygraph for recording intramammary pressure and arterial blood pressure respectively.

Two subcutaneous electrodes in the skin overlying the chest were connected to another channel of the Polygraph and the amplified electrocardiogram was monitored by an audio system and by a digital counter (SA 535, Racal Instruments Ltd.). The hormone preparations used were synthetic oxytocin (Pitocin, Parke, Davis & Co.) and highly purified natural arginine vasopressin (Tonephin, Farbwerke Hoechst, A. G.) and these were administered through the jugular cannula.

The stimulating electrode consisted of a size 00 stainless steel insect pin (Clay Adams Inc.) soldered to a length of 24-gauge stainless steel tubing and it was insulated with epoxy resin except for 0·2 mm. at the tip. The electrode was lowered into the brain using stereotaxic co-ordinates (Sawyer et al. 1954; J. S. Tindal, G. S. Knaggs & A. Turvey, unpublished observations) and monopolar stimulation was applied between the tip of the electrode and an indifferent electrode in the scalp, using square-wave pulses (50/sec., 1 msec. duration, 250–260 μA, 3 v) for a period of 30 sec. at each site. The electrode was lowered within the brainstem in steps of 0·5 mm. and up to nine vertical tracks were explored in individual experiments. Responsive sites were marked by passing a 10 μA d.c. current through the electrode system for 1 min. and at the end of the experiment the head was perfused, as described for the guinea-pig (Tindal et al. 1967b), to develop the Prussian Blue reaction at lesion sites and to fix the brain. After fixation, serial transverse sections, 80 μ thick, were cut through the region of the brain containing electrode tracks and were stained with Neutral Red. Stimulation sites were determined microscopically and were plotted on drawings of transverse sections of rabbit brain from posterior 10 mm. to anterior 1 mm. at 1-mm. intervals. For reasons of clarity, all sites were plotted on the right-hand side of the brain.
Results

Release of oxytocin was detected after electrical stimulation of the brain by monitoring intramammary pressure and the amount released was estimated approximately by a comparison with milk-ejection responses evoked by i.v. injection of synthetic oxytocin (Fig. 1). Since vasopressin has inherent milk-ejection activity, it was necessary to determine whether stimulation-induced milk-ejection responses were caused by release of oxytocin, vasopressin, or by a mixture of the two hormones. This was done by recording arterial blood pressure in addition to intramammary pressure. Thus, if a milk-ejection response had been caused in part or in whole by substantial release of vasopressin, this would manifest itself by a simultaneous rise in arterial blood pressure, which could then be compared with the effect of i.v. injection of arginine vasopressin.

Fig. 1. Polygraph record of intramammary pressure and arterial blood pressure showing response to i.v. injection of synthetic oxytocin, natural vasopressin and electrical stimulation of the ascending path for oxytocin release in the brain of the anaesthetized lactating rabbit. Vertical arrows denote i.v. injection of 2 m-u. synthetic oxytocin (oxy), 2 m-u. natural arginine vasopressin (v) or 0·2 ml. of 0·9 % NaCl solution (s). The horizontal bar denotes a 30-sec. period of electrical stimulation (see text for details) at one of the sites clustered together near the medial geniculate body (Fig. 2d). Note release of oxytocin without detectable release of vasopressin.

The threshold dose for the milk-ejection response was within the range 0·5–1·0 m-u. oxytocin i.v. and stimulation-induced milk-ejection responses which were equivalent to less than 1·0 m-u. oxytocin were ignored. The amount of oxytocin released after stimulation of the pathway varied from 1 to 6 m-u. However, when stimulating the basal hypothalamus, as in a previous study in the guinea-pig, we reduced the time of stimulation from 30 to 3 sec. to avoid causing the release of excessive amounts of oxytocin, which can lead to prolonged tachyphylaxis of the mammary gland, and hence to the premature termination of the experiment (see Tindal et al. 1967b).

Release of oxytocin occurred after electrical stimulation of a discrete region of the
Fig. 2. For legend see facing page.
lateral mesencephalic tegmentum at planes P 10, 9 and 8 (Fig. 2a, b, c), while further forwards the responsive area lay medio-ventral to the medial geniculate body (planes P 8, 7 and 6, Fig. 2c, d, 3a). Passing rostrally, responsive sites were found in association with the extreme rostral central grey matter (planes P 6, 5 and 4, Fig. 3a, b, c), in the subthalamus (planes P 5, 4 and 3, Fig. 3b, c, d), and in the posterior hypothalamus they were clustered together just lateral to the mammillo-thalamic tract (plane P 2, Fig. 4a). When the more rostral planes were explored, close to the hypothalamic paraventricular nucleus, electrical stimulation of many sites in the hypothalamus elicited release of oxytocin (planes APO and A 1, Fig. 4c, d).

The threshold dose for the pressor response was within the range 1–2 m-u. and for the milk-ejection response 4–5 m-u. arginine vasopressin i.v. Release of vasopressin was detected in several instances, both with or without an accompanying release of oxytocin. In plane P 8 (Fig. 2c) a release of vasopressin alone (equivalent to 2 m-u.) occurred after stimulation of a site just lateral to the medial longitudinal fasciculus. Further rostrally, releases of vasopressin alone occurred after stimulation of a site in the subthalamus (equivalent to 2 m-u.) (plane P 6, Fig. 3a), and after stimulation of two sites more dorsally placed in the reticular formation (equivalent to 4 and 2 m-u., plane P 5, Fig. 3b). At plane P 3, stimulation of two adjacent sites in one track evoked release of vasopressin alone (equivalent to 4 m-u.) from the upper site (denoted by black triangle in Fig. 3d) and 4 m-u. vasopressin plus 1 m-u. oxytocin from the lower one (the black triangle denoting this site is almost obscured by the cluster of positive sites for oxytocin release which surround it). In the hypothalamus, a mixture of oxytocin and vasopressin was released after stimulation of three sites at plane A 1 on top of the optic tract (4 m-u. oxytocin plus 4 m-u. vasopressin, 2 m-u. oxytocin plus 5 m-u. vasopressin and 1.5 m-u. oxytocin plus 5 m-u. vasopressin, from medial to lateral sites respectively, Fig. 4d). Stimulation of a site in the tuberal region (plane APO, Fig. 4c) elicited release of oxytocin in excess of 2 m-u. and vasopressin in excess of 5 m-u. (based on responses to i.v. injection of these doses of the two hormones before stimulation). However, a 30-sec. period of stimulation was accidentally used here instead of the more appropriate 3-sec. period for this region, and the resulting large release of oxytocin rendered the mammary gland insensitive to oxytocin, which terminated the experiment.

Fig. 2. The ascending path of the milk-ejection reflex in the rabbit. Drawings of transverse sections through the brain showing sites where electrical stimulation evoked release of oxytocin (solid circles), of vasopressin or vasopressin plus oxytocin (solid triangles; see text for description of releases relating to individual sites) or no release of oxytocin or vasopressin (open circles). Planes are labelled as mm. posterior (P) or anterior (A) to the anterior-posterior zero plane (APO) passing rostrally from Fig. 2a through to Fig. 4d. See text for details. Note the compact ascending path of the milk-ejection reflex in the mid-brain. See Fig. 1 for record of milk-ejection response after electrical stimulation of one of the sites clustered in a triangle in d. Abbreviations: CG = central grey; MG = medial geniculate body; MLF = medial longitudinal fasciculus; MT = mammillo-thalamic tract; OT = optic tract; PV = paraventricular nucleus; STH = subthalamus.
Fig. 3. The ascending path of the milk-ejection reflex in the rabbit. See legend to Fig. 2 for details. As the path enters the diencephalon it bifurcates into dorsal and ventral paths.
Fig. 4. The ascending path of the milk-ejection reflex in the rabbit. See legend to Fig. 2 for details. The dorsal and ventral paths reunite in the posterior hypothalamus, while further rostrally the pathway intermingles with efferent fibres from the paraventricular nucleus, from which it cannot be distinguished by a simple stimulation technique.
DISCUSSION

The ascending pathways for release of oxytocin in the brainstem of the rabbit appear to be essentially the same as those found previously in the guinea-pig (Tindal et al. 1967a, b). There is a single ascending path on each side of the mesencephalon lying in the lateral tegmentum. Further rostrally, the pathway on each side divides into a dorsal path, passing forwards in association with the extreme rostral central grey matter, and a ventral path passing through the subthalamus. These pathways, on each side of the brain, reunite in the posterior hypothalamus, but as they ascend through the hypothalamus the picture is complicated by the efferent tracts passing out of the paraventricular nucleus and sweeping down to the pituitary stalk. A simple stimulation technique obviously cannot distinguish one from the other, and this portion of the pathway is now being investigated in the guinea-pig by a combination of stimulation and surgical techniques (J. S. Tindal & G. S. Knaggs, unpublished work).

The pathway in the lateral tegmentum of the mid-brain appears to be even more compact than in the guinea-pig, and since at this level of the brain electrical stimulation elicited release of oxytocin only when the electrode tip was in this discrete path and nowhere else, it is believed that sensory information evoked by the suckling stimulus is carried to the hypothalamus by the spinothalamic system of fibres, which relay with other ascending systems in the diencephalon. The anatomical and physiological evidence behind this reasoning has already been reviewed (Tindal, 1967; Tindal et al. 1967b) and will not be repeated here. Although Holland, Woods & bulbsbrook (1963) reported milk-ejection responses in the rabbit after stimulation of the lemniscal system at the level of the medulla, our present and previous findings (Tindal et al. 1967b) suggest that the lemniscal system plays only a minor role in the milk-ejection reflex. In the diencephalon, the present findings concur with the view of Rothballer (1966) that ascending pathways from the periaqueductal grey and paraventricular system and from the subthalamus are implicated in the release of oxytocin. Although stimulation of many structures in the forebrain has been reported to elicit release of oxytocin, there is no evidence at present to suggest their involvement in reflex release of the hormone, unless, as suggested in the review by Cross (1966), they are concerned with facilitation or conditioning of the release of oxytocin.

The present results also support the previous finding in the guinea-pig that the afferent path of the milk-ejection reflex is a pathway for the preferential release of oxytocin from the neurohypophysis and is not a major pathway for vasopressin release (Tindal, Knaggs & Turvey, 1968). Definite evidence of vasopressin release was obtained after stimulation of only ten sites. In the hypothalamus, one was found in the tuberal region and 3 above the optic tract, and the combined release of oxytocin and vasopressin associated with these sites presumably reflected activation of efferent fibres from both the supraoptic and paraventricular nuclei. In the mesencephalon, release of vasopressin alone was evoked by stimulation of five sites in the reticular formation and subthalamus, which agrees with results in the dog and monkey (Mills & Wang, 1964; Hayward & Smith, 1964). Stimulation of one site in the subthalamus, immediately dorsal to the ventral path for oxytocin release, caused the release of both oxytocin and vasopressin, whereas stimulation of the sites 0-5 mm. dorsal or 0-5 mm.
ventral to this site elicited release of vasopressin or oxytocin, respectively, suggesting that at this point alone in the mid-brain the ascending paths for release of oxytocin and vasopressin approach one another.

Finally, although the stimulation technique has traced what is probably the main ascending pathway for oxytocin release in the mid-brain, the possibility still remains that the type of punctate stimulation used in the present work may not reveal other, more diffuse, ascending systems. This must be investigated by placing bilateral lesions in the compact ascending path in the mesencephalon of the lactating animal and determining whether or not the natural milk-ejection reflex consequent to suckling is blocked.

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