J. Pharm. Pharmac., 1974, **26**, 193–196 *Received June* 8, 1973

Bioassay of vasopressin on the rabbit isolated urinary bladder

L. ANGELUCCI, G. FALCONIERI ERSPAMER AND L. NEGRI*

Institute of Pharmacology and Pharmacognosy and Institute of Medical Pharmacology I, University of Rome, Città universitaria, I-00185 Rome, Italy*

The rabbit isolated urinary bladder contracted in the presence of Lys^8-vasopressin from threshold concentrations of 5 to 30 μU ml^{-1} (0·02 to 0·11 ng ml^{-1}). Responses were proportional to the dose used. The tissue gave satisfactory results either fresh or after storage for several days in cold Tyrode or Krebs solution (3–4°). The preparation also contracted in the presence of oxytocin, but it was 7 to 20 times less sensitive to this peptide. A number of other peptides and amines known to stimulate smooth muscle showed low activity on the rabbit urinary bladder and, occasionally, intense tachyphylaxis.

In the course of a systematic study on the effects of active biogenic peptides on extravascular smooth muscle, it was observed that the rabbit isolated urinary bladder was highly sensitive to vasopressin and that there was a correlation between the dose given and the effect (Falconieri Erspamer, Negri & Piccinelli, 1973).

We now describe the possible use of the rabbit urinary bladder in the bioassay of vasopressin and the relative potency of several other naturally occurring agents known to stimulate the isolated smooth muscle.

METHODS AND MATERIALS

Bioassay. The mucosal layer was carefully removed from longitudinal strips of the rabbit isolated urinary bladder and the muscle strips were suspended in 10 ml of aerated Krebs or Tyrode solution at 37°.

The motility of the preparation was recorded on a smoked drum with an isometric microdynamometer (7001, U. Basile, Milan), using a DY 2 strain-gauge transducer (force up to 10 g). A dose cycle of 15 to 25 min was used, the drugs being allowed to act for 4 min.

Drugs. The standard vasopressin preparation used in all the experiments was synthetic Lys^8-vasopressin (Sandoz). Other drugs used were: synthetic bradykinin and oxytocin (Sandoz); synthetic eledoisin, physalaemin, caerulein and bombesin (Farmitalia); synthetic Val^5-angiotensin II-Asp-β-amide (Ciba); synthetic substance P (Beckman); prostaglandins E_1 and $F_{1\alpha}$ (The Upjohn Co.); 5-hydroxytryptamine creatinine sulphate and histamine dihydrochloride.

According to Boissonas, Guttman & others (1961), 1 mg of Lys^8-vasopressin corresponds to 270 units (1 unit = 3·7 μg), and 1 mg of oxytocin to 450 units (1 unit = 2·2 μg).

RESULTS

The threshold dose of vasopressin required in more than 30 experiments varied from 5 to 30 μU ml^{-1} (0·02 to 0·11 ng ml^{-1}). It was approximately the same in fresh

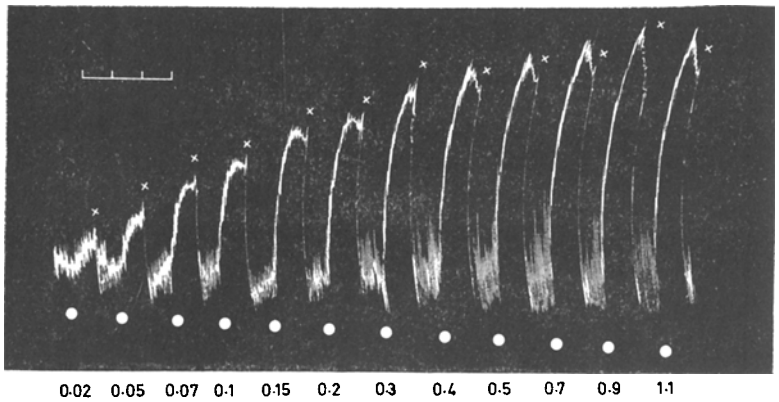

FIG. 1. Rabbit urinary bladder preparation suspended in 10 ml of Tyrode solution, at 37°. Response produced by increasing doses of vasopressin (doses in mU ml^{-1}). At x, washing. Time marks: 4 min.

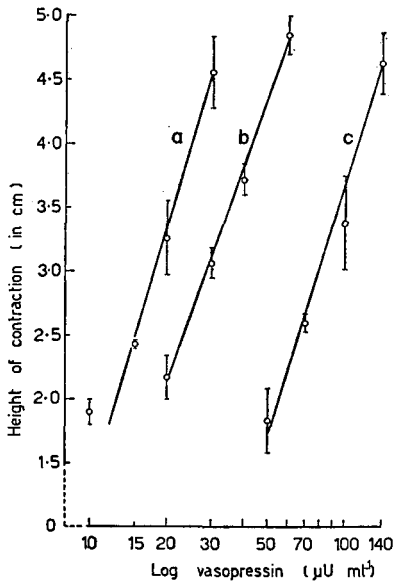

FIG. 2. Rabbit urinary bladder preparation. Three dose-response curves of contractions elicited by vasopressin. Each point is the mean of four assays, the vertical bars being standard errors.

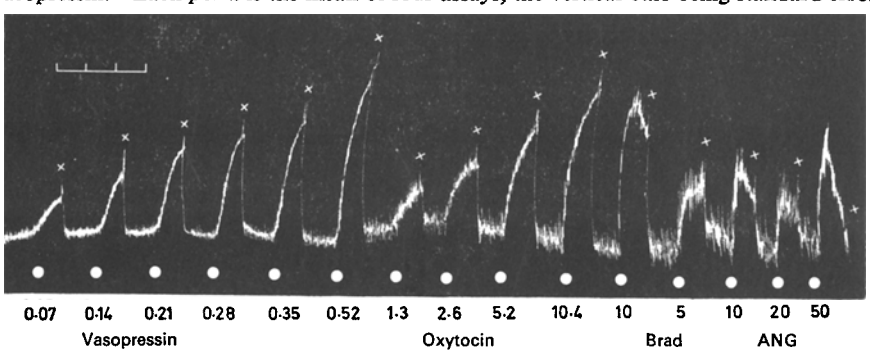

FIG. 3. Rabbit urinary bladder preparation (kept in cold Krebs solution for 3 days) suspended in 10 ml of Krebs solution, at 37°. Responses produced by different doses of vasopressin, oxytocin, bradykinin (Brad) and angiotensin (ANG). All doses in ng ml^{-1}. At x, washing. Time marks: 4 min. In this experiment vasopressin was approximately 15 times more potent than oxytocin and 20–25 times more potent than bradykinin. Tachyphylaxis was evident with angiotensin.

strips of urinary bladder and in strips kept in Tyrode or Krebs solution at 3–4° for 1 to 4 days. Thereafter sensitivity declined, but concentrations of 50–100 μU ml^{-1} were still effective after 5 to 7 days. Halving the concentration of calcium in the Tyrode solution reduced the sensitivity of the preparation and reduction of the bath temperature to 30–32° had a similar effect.

After administration of the peptide there was a short latent period and the contraction developed progressively so that, to allow exact dose assays, the contact time should be kept rigorously constant. In our experiments, 4 min was a suitable contact time. After the tissue had been washed, its tone returned to basal levels in 3 to 12 min, depending on the dose. Tachyphylaxis did not occur at an interval between doses of at least 10 min.

The relation between dose and effect is shown in Fig. 1 and Fig. 2 shows a dose assay of 4 different doses of vasopressin. Ratios of the standard deviations of single responses to the slope of the regression lines varied from 0·01 to 0·09 in six experiments. These ratios are an inverse measure of the precision of an assay and should lie under 0·05 for a satisfactory assay (Holton, 1948).

Comparison of the effects, produced in more than 20 assays by vasopressin and oxytocin, showed that the rabbit urinary bladder was 7 to 20 times more sensitive to vasopressin than to oxytocin (Fig. 3). This calculation was based on the unitage per mg of oxytocin and Lys^{8}-vasopressin mentioned earlier. If the activity of vasopressin is taken to be 100, then the relative activities of other biogenic compounds known to stimulate smooth muscle were: eledoisin <2, physalaemin <2, substance *P* <0·05, bradykinin 1–5, caerulein <0·05, bombesin <0·05, angiotensin 1–10 (evident tachyphylaxis), prostaglandin E_1 <1, prostaglandin $F_{1\alpha}$ <(—)-histamine <2, 5-hydroxytryptamine 0·2–1. Atropine (0·1–0·5 μg ml^{-1}) did not appreciably affect the response of the tissue to vasopressin.

DISCUSSION

Botting (1964) found that an isolated preparation of the proximal portion of the guinea-pig colon contracted in the presence of vasopressin in concentrations of 1 to 1000 μU ml^{-1}, and that there was a good dose-response relation for the polypeptide. The tissue was 18 times less sensitive to oxytocin. Present results show that the rabbit isolated urinary bladder, like the guinea-pig colon, is highly and selectively sensitive to vasopressin. Although the sensitivity was somewhat less for the urinary bladder (5 μU ml^{-1}) than for the guinea-pig colon (1 μU ml^{-1}), there was less variation in the threshold dose with the former preparation (5 to 30 μU ml^{-1} compared with 1 to 1000 μU ml^{-1} for guinea-pig colon). Moreover, the urinary bladder gave satisfactory results after storage for 2–4 days in cold bath fluid, so that numerous assays could be carried out in the same preparation on different days.

The rabbit urinary bladder also contracted in the presence of oxytocin, but was 7 to 20 times less sensitive to this polypeptide. The other natural peptides and amines known to stimulate smooth muscle had little effect on the rabbit urinary bladder. Only bradykinin and angiotensin presented 1 to 5% and 1 to 10%, respectively, of the activity of vasopressin, but tachyphylaxis was frequently found with angiotensin.

The rabbit urinary bladder may therefore be included among the isolated smooth muscle preparations which can be used in the bioassay of vasopressin and in the

discrimination of this polypeptide from other biogenic compounds active on smooth muscle.

Acknowledgement

This work was supported by grants from the Consiglio Nazionale delle Ricerche, Rome.

REFERENCES

BOISSONAS, R. A., GUTTMAN, S., BERDE, B. & KONZETT, H. (1961). *Experientia*, **17**, 377–390.
BOTTING, J. H. (1964). *Br. J. Pharmac. Chemother.*, **24**, 156–162.
FALCONIERI ERSPAMER, G., NEGRI, L. & PICCINELLI, D. (1973). *Arch. Pharmac.*, **279**, 61–74.
HOLTON, P. (1948). *Br. J. Pharmac, Chemother.*, **3**, 328–334.