Neurohypophyseal effects of angiotensin: further studies

D. J. GAGNON*, P. SIROIS, *Département de Physiologie et Pharmacologie, Centre Hospitalier Universitaire, Université de Sherbrooke, Sherbrooke, Québec, Canada*

Angiotensin II has been reported to stimulate the release of vasopressin (Gagnon, Cousineau & Boucher, 1973) and the accumulation of cyclic AMP (Gagnon & Heisler, 1974) in rat neurohypophyses and numerous analogues of angiotensin II were able to produce similar neurohypophyseal effects (Gagnon, Sirois & Boucher, 1975a; Gagnon, Sirois & Park, 1975b). It has also been demonstrated that the neurohypophyseal effects of angiotensin I were most likely due to its conversion into angiotensin II (Sirois & Gagnon, 1975) by local converting enzyme (Yang & Neff, 1972, 1973). These conclusions were based on the fact that the decapeptide was ineffective in the presence of an inhibitor of the converting enzyme (SQ 20881) which had been shown not to affect peripheral responses to angiotensin (Ng & Vane, 1970; Engel, Schaeffer & others, 1972). This did not exclude the possibility that neurohypophyseal receptors to angiotensin might have been influenced by SQ 20881, and the present experiments were made to resolve this.

Rat isolated neural lobes (halved) were used according to Gagnon & others (1975a). After this incubation in 1 ml phosphate buffer (pH 7·4) at 37° under 5% CO_2 in oxygen, the medium was removed for assay and the lobes were homogenized in 0·25% acetic acid, heated, centrifuged and stored at 4° until assayed. The release of vasopressin into the medium was expressed as the percentage of the total amount (medium and tissue) present in the incubated glands. Vasopressin was assayed according to Gilmore & Vane (1970) with minor modifications (Cousineau, Gagnon & Sirois, 1973).

For cyclic AMP studies, halved glands were incubated in the presence of theophylline (10 mM) at 37° in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7·4) (Gagnon & Heisler, 1974). After incubation, the tissue was immediately homogenized and the protein concentration was determined in a 200 μl aliquot (Lowry, Rosebrough & others, 1951). The extracted cyclic AMP was finally measured in 50 mM acetate (pH 5·0) according to Tsang, Lehotay & Murphy (1972). Results are expressed in terms of concentration (pmol mg^{-1} tissue protein). SQ 20881 has the following structure: pyro Glu-Trp-Pro-Arg-Pro-Glu-Ile-Pro-Pro, and was kindly supplied by Squibb Institute, U.S.A. Angiotensin II used in this study is hypertensin, Ciba. All agents were prepared freshly and added to the incubation medium in a volume of 10 μl.

Our aim was to find out if SQ 20881 was capable of influencing angiotensin II-induced stimulation of vasopressin release and cyclic AMP accumulation in rat neural lobes. For this purpose, glands were preincubated (25°) with or without SQ 20881 (1×10^{-6} M) for 10 min, followed by an incubation (10 min) in the presence or in the absence of angiotensin (1×10^{-9} M). As indicated in Fig. 1, in control glands, the accumulation of cyclic AMP averaged 600 ± 94 pmol mg^{-1} tissue protein, and the mean vasopressin release in the medium was 9·8 ± 0·7% after incubation. When angiotensin was added to the medium at the beginning of the incubation period, the tissue content of the cyclic nucleotide increased to 903 ± 108 pmol mg^{-1} tissue protein. Similar results were obtained with vasopressin release which increased to 13·8 ± 1·4% in response to the octapeptide. The values obtained for both neurohypophyseal events were significantly higher in the presence of angiotensin than those observed in control glands ($P < 0.05$).

Fig. 1 also indicates that the converting enzyme inhibitor did not influence significantly the accumulation of cyclic AMP which showed mean levels of 606 ± 59 pmol mg^{-1} tissue potein. However, under the same conditions the spontaneous release of vasopressin was slightly increased, although not significantly, to mean values of 12·1 ± 1·0%. When angiotensin wad added to the medium containing glands that had been preincubated in the presence of the converting enzyme inhibitor, the natural octapeptide induced its usual neurohypophyseal effects; cyclic AMP content was

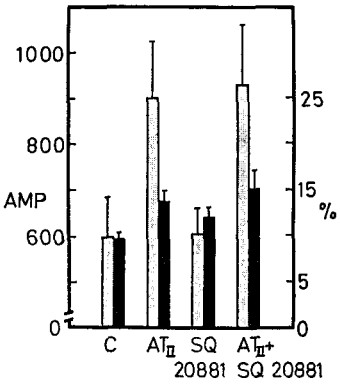

FIG. 1. Cyclic 3′,5′-AMP concentrations (p mol mg^{-1} protein), left hand ordinate and stippled columns (n = 5) and vasopressin release (%) right hand ordinate and solid columns (n = 6) in: control glands (C), in glands incubated in the presence of either angiotensin II (ATII) (1×10^{-9} M) or the inhibitor of converting enzyme (SQ 20881) (1×10^{-6} M) and finally in the presence of both SQ 20881 (1×10^{-6} M) and ATII (1×10^{-9} M). Vertical bars represent means ± s.e.m. n = number of experiments.

* Correspondence and present address: Département de Pharmacologie, Faculté de Médecine, Université Laval, Québec, P.Q. Canada. G1K 7P4.

increased to 893 ± 104 pmol mg^{-1} tissue protein, whereas the mean vasopressin release reached 15·1 ± 1·8%. These values were significantly higher ($P < 0.05$) than those observed in control glands, but were not different from those obtained with glands incubated in the presence of angiotensin II alone.

Our previous findings that inhibition of the converting enzyme by SQ 20881 could prevent the increase in cyclic AMP content and vasopressin release induced by angiotensin I indicated that the decapeptide was probably transformed into an octapeptide (angiotensin II) before producing its neurohypophyseal effects (Sirois & Gagnon, 1975). It was then assumed that this inhibitory effect was not due to a direct antagonism of the neurohypophyseal receptor site for angiotensin by SQ 20881, as evidenced by Ng & Vane (1970) and Engel & others (1972) who demonstrated that the nonapeptide did not affect the response to angiotensin mediated by peripheral receptor sites. The present results demonstrated clearly that SQ 20881 does not interfere with the binding of angiotensin to its neurohypophyseal receptors. They also provided further evidence as to the role of the converting enzyme in the neurohypophyseal actions of angiotensin I.

We wish to thank Mr P. Boucher for his excellent technical assistance. This work was supported by grants from the Medical Research Council of Canada and the Quebec Heart Foundation.

February 27, 1976

REFERENCES

COUSINEAU, D., GAGNON, D. J. & SIROIS, P. (1973). *Br. J. Pharmac.*, **47**, 315–324.
ENGEL, S. L., SCHAEFFER, I. R., GOLD, B. I. & RUBIN, B. (1972). *Proc. Soc. exp. Biol. Med.*, **140**, 240–244.
GAGNON, D. J., COUSINEAU, D. & BOUCHER, P. J. (1973). *Life Sci.*, **12**, 487–497.
GAGNON, D. J. & HEISLER, S. (1974). *Biochim. biophys. Acta*, **338**, 394–397.
GAGNON, D. J., SIROIS, P. & BOUCHER, P. J. (1975a). *Clin. exp. Pharmac. Physiol.*, **2**, 305–313.
GAGNON, D. J., SIROIS, P. & PARK, W. K. (1975b). *Ibid.*, **2**, 315–322.
GILMORE, N. J. & VANE, J. R. (1970). *Br. J. Pharmac.*, **38**, 633–652.
LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDAL, R. J. (1951). *J. biol. Chem.*, **193**, 265–275.
NG, K. K. F. & VANE, J. R. (1970). *Nature, Lond.*, **225**, 1142–1144.
SIROIS, P. & GAGNON, D. J. (1975). *J. Neurochem.*, **25**, 727–729.
TSANG, C. P. W., LEHOTAY, D. C. & MURPHY, B. E. P. (1972). *J. clin. Endocr. Metab.*, **35**, 809–817.
YANG, H. Y. T. & NEFF, N. H. (1972). *J. Neurochem.*, **19**, 2443–2450.
YANG, H. Y. T. & NEFF, N. H. (1973). *Ibid.*, **21**, 1035–1036.

Comparison of the dopaminergic effects of *N*-substituted aporphines

M. K. MENON, W. G. CLARK†, J. G. CANNON*, *Psychopharmacology Research Laboratory, Veterans Administration Hospital, Sepulveda, California 91343, Department of Biological Chemistry, University of California Centre for Health Sciences, Los Angeles and *Division of Medicinal Chemistry and Natural Products, University of Iowa, College of Pharmacy, Iowa City, Iowa, U.S.A.*

Because apomorphine has short duration of action, it causes depression and is ineffective on oral administration with development of azetomia on increasing its dose, its therapeutic usefulness (Cotzias, Lawrence & others, 1972) in alleviating Parkinsonian symptoms is limited. The possibility of the development of long-acting analogues with minimal emetic effects and longer duration of dopaminergic effects than the parent drug, still exists. Since earlier studies of many investigators (Koch, Cannon & Burkman 1968; Pinder, Buxton & Green 1971; Lal, Sourkes & others, 1972; Neumeyer, McCarthy & others, 1973a; Neumeyer, Neustadt & others, 1973b; Neumeyer, Granchelli & others, 1974; Saari & King, 1974) showed that changes in ring substitution reduced or abolished the dopaminergic effects of the parent compound, we selected 4 apomorphine derivatives differing from apomorphine only in their *N*-substituent. Activation of reserpinized mice was used as the criterion for evaluation of their dopaminergic effects and apomorphine was used as the reference standard. Since one of the main drawbacks of apomorphine is its emetic effect, its interaction with two antiemetics also has been investigated.

Male Swiss mice (23–28 g, Horton Labs) maintained at 23 ± 1° were pretreated with reserpine (5 mg kg^{-1}) 4 h before the experiment. Various doses of the apomorphines were administered and immediately after injection, the amounts were placed individually in plastic chambers (12·5 cm^3) on an activity meter (Model 2S, Columbus Instrument Company, Ohio) and their motor activity was recorded. The aporphines tested were the hydrochlorides of (—)-apomorphine, (±)-norapomorphine, (±)-ethylnorapomorphine, (±)-propylnorapomorphine and (±)-methylcyclopropylnor-

† Correspondence.