given food on alternate days, and water was supplied on a continuous basis. Recordings were made only during periods when the rat was quiet.

At the end of the recording period, each rat was sacrificed with a blow to the head; the telemetry system was checked for proper placement of the transducers, the tissue reaction observed, the fundic area of the stomach immediately removed and rapidly frozen at $-70\,^{\circ}C$ until thawing for cyclic nucleotide assay. A double antibody radioimmunioassay kit procedure (Colabrative Research Inc., Waltham, Mass., USA) for cAMP and cGMP was performed on the fundic tissue[8]. Labelled nucleotide recovery was 72.3% on duplicate samples. Displacement of radioactive-labeled antigen by unlabeled cAMP or cGMP standards is linear when plotted as a semi-logarithmic function. The standard curves for cAMP and cGMP are reproduced in the Figure.

Ten recordings per rat were randomly selected, and a motility index[9] was calculated for each rat. Concentration of cyclic nucleotides was calculated using duplicate samples from each rat and converted into picomoles/g wet wt., tissue. Student's t-test was used to determine significance ($p < 0.05$). Significant differences were found ($p < 0.05$) between the two groups for all three factors: motility index, concentration of cAMP, concentration of cGMP (Table).

Previous investigators[10] had established a relationship between PGE_1, decrease in cAMP levels, increase in cGMP tissue levels and increased motility in smooth muscle studied in vivo. The increased motility observed in this study was attributed to the action of exogenous PGE_1 on the force and contractility of the circular and longitudinal fundic muscle as well as enhancing the effect of endogenous mucosal PGE_1.

The presence of exogenous PGE_1 administered by the i.p. route, served to influence total fundic tissue levels of the two cyclic nucleotides and served as a positive feedback on cholinergic activity. The increase or decrease in contractile activity was affected by the interaction between exogenous PGE_1, cAMP and cGMP and controlled by significant decreases in the tissue levels of these two cyclic nucleotides.

Motility index and cyclic Nucleotide values

	Motility index (Mean ± SEM)[b]	cAMP[a] (Mean ± SEM)[b]	cGMP[a] (Mean ± SEM)[b]
Control	61 ± 16.10	16 ± 1.40	0.30 ± 0.02
PGE_1 injected	84 ± 8.20	5 ± 1.20	0.45 ± 0.03

[a] Data are expressed as picomoles of cyclic nucleotide/g fundic tissue. wet. wt. [b] Each value is the mean (± SEM) of 5 different tissues.

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Effects of Three Synthetic Peptides Analogous to Neurohypophyseal Hormones on the Excitability of Giant Neurones of *Achatina fulica* Férussac

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Summary. Deamino-dicarba-(D-D-)oxytocin and D-D-Arg-vasotocin at 10^{-4} kg/l showed an excitatory effect on the periodically oxcillating neurone (PON) of *Achatina fulica* Férussac. D-D-Arg-vasopressin had no effect.

We obtained three synthetic peptides analogous to neurohypophyseal hormones, deamino-dicarba-oxytocin (D-D-oxytocin), deamino-dicarba-Arg-vasotocin (D-D-Arg-vasotocin) and deamino-dicarba-Arg-vasopressin (D-D-Arg-vasopressin) in the analytically pure state[2,3]. In the present study, we attempted to compare the effects of these peptides on the excitability of 2 spontaneously firing giant neurones (the PON, periodically oscillating neurone; and the TAN, tonically autoactive neurone)[4–6] identified in the subesophageal ganglia of an African giant snail, *Achatina fulica* Férussac.

A micropipette, implanted into one of the identifiable neurones, recorded its intracellular biopotential with a pen-writing galvanometer, and counted the number of its spike discharges per min by a spike counter. We applied these peptides dissolved in the snail's physiological solution[7] directly to the dissected ganglia (bath application). We also applied these peptides locally to an identifiable neurone (microdrop application)[8]. In this case, we made a microdrop in the open air (about 150 µm in diameter) of a peptide solution at the tip of a micropipette containing the peptide solution by oil pressure, and placed the microdrop on the surface of an identifiable neurone (its diameter is about 200 µm). As the electrical resistance of the neuromembrane, we measured its current-voltage relationships (I–V curve), using 2 microelectrodes implanted into the soma: one was to record the biopotential, the other to apply the transmembrane triangular current of long duration. We always recorded

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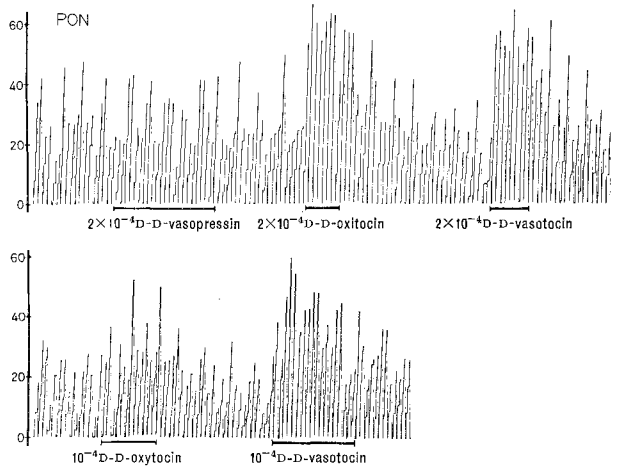

Fig. 1. Effects of 3 synthetic peptides analogous to neurohypophyseal hormones, D-D-Arg-vasopressin, D-D-oxytocin and D-D-Arg-vasotocin, on PON excitability (bath application). The 2 traces were recorded continuously. Ordinate: the number of spike discharges/min. Abscissa: time course, each histogram is 1 min. D-D-oxytocin and D-D-Arg-vasotocin even at 10^{-4} kg/l clearly showed an excitatory effect on the PON, though D-D-Arg-vasopressin at 2×10^{-4} kg/l was ineffective.

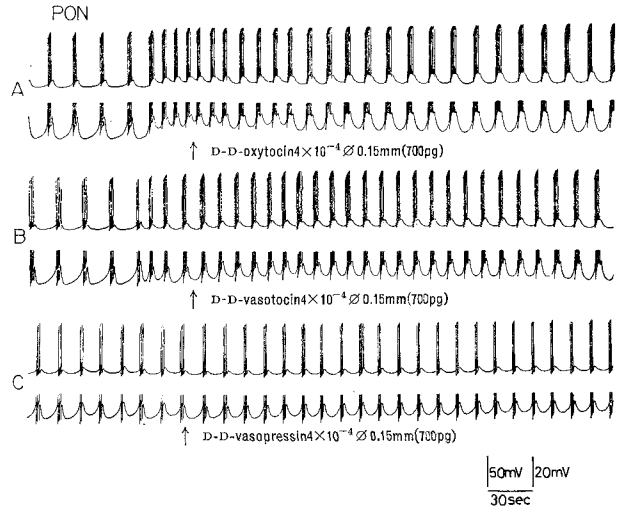

Fig. 2. Effects of 3 synthetic peptides analogous to neurohypophyseal hormones on the PON biopotential (microdrop application). All traces (A, B and C) have been recorded from the same PON. Upper traces of A, B and C are full-spike recordings of the PON biopotential. Lower traces of A, B and C are high gain recordings of upper traces. In lower traces, spike peaks have been cut by an electronic voltage clipper. We applied a microdrop (about 150 µm in diameter) of 4×10^{-4} kg/l D-D-oxytocin (total amount of this substance is estimated to be 700 pg) in A (arrow), a microdrop of 4×10^{-4} D-D-Arg-vasotocin in B (arrow), and a microdrop of 4×10^{-4} D-D-Arg-vasopressin in C (arrow). Left vertical bar: calibration for upper traces (50 mV). Right vertical bar: calibration for lower traces (20 mV). Horizontal bar: time course (30 sec). Note that a microdrop of D-D-oxytocin and D-D-Arg-vasotocin caused depolarization of the PON neuromembrane and augmentation of the number of its spike discharges, although D-D-Arg-vasopressin, applied in the same way, showed no effect.

the biopotential shift and the intensity of the applied current with an oscilloscope. When two I–V curves, one measured in the physiological state and the other under the presence of some peptide, were superimposed, the recordings of the applied current intensity were graphically erased after confirming their fairly triangular shapes (no rectification of the microelectrode to apply the current).

Experimental results obtained in the present study are summarized in the Table. In Figure 1, the effect of these peptides administered by the bath application on PON excitability is compared. Although D-D-Arg-vasopressin at 2×10^{-4} kg/l showed no effect on the PON (periodically oscillating neurone), D-D-oxytocin and D-D-Arg-vasotocin in the same concentration had a remarkable excitatory effect. The latter two substances at even 10^{-4} kg/l clearly showed excitatory effects on this neurone.

As Figure 2, A and B shows, the application of a microdrop of D-D-oxytocin and of D-D-Arg-vasotocin (the total amount of each applied peptide is calculated to be about 700 pg) (arrow) caused, with a short latency, a clear depolarization of the PON neuromembrane and an augmentation of its spike frequency without synaptic noises.

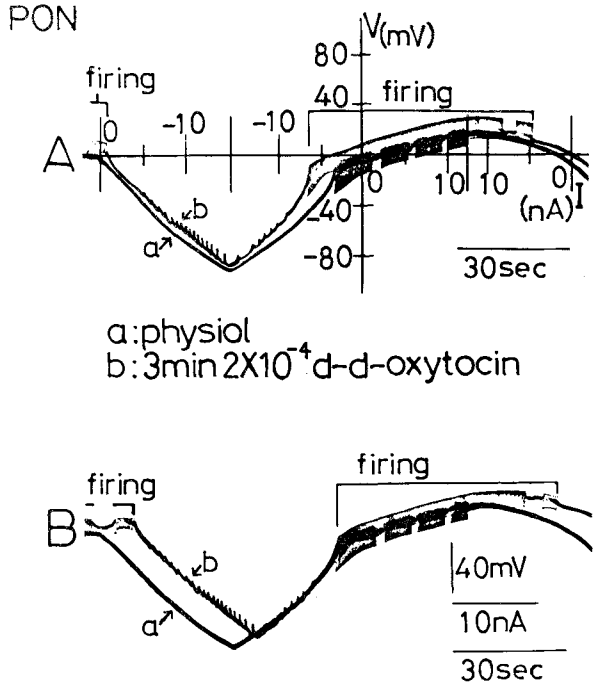

Fig. 3. Changes of the current voltage relationships (I–V curve) of the PON neuromembrane, caused by D-D-oxytocin (bath application). To measure the I–V curve, a triangular current (hyperpolarizing, depolarizing and hyperpolarizing $(1.2 \times 10^2)^{-1}$ Hz) has been applied into the PON soma. a) PON I–V curve measured in the physiological state. b) PON I–V curve measured 3 min after 2×10^{-4} kg/l D-D-oxytocin. In A) the two I–V curves (a and b) have been superimposed using the initial polarization level (just before the triangular current application) as the common standard. Ordinate (V): voltage of biopotential shift (mV). Abscissa (I): intensity of applied current (nA). Horizontal bar: time course (30 sec). In B) the two I–V curves (a and b) have been superimposed using the firing level as the common standard. Vertical bar: calibration of biopotential shift (40 mV). Upper horizontal bar: intensity of applied current (10 nA). Lower horizontal bar: time course (30 sec). Note that the two I–V curves (one measured in the physiological state, the other measured in the presence of 2×10^{-4} kg/l D-D-oxytocin) are concordant in the wide range of membrane polarization level, when the 2 curves have been superimposed using the firing level as the common standard.

Effects of 3 synthetic peptides analogous to neurohypophyseal hormones (obtained in the analytically pure state from Protein Research Foundation, Osaka) on the excitability of 2 identifiable giant neurones (the PON, periodically oscillating neurone; and the TAN, tonically autoactive neurone) of *Achatina fulica* Férussac (bath application)

Substance	Amino acid sequence	Concentration (kg/l)	Effect on PON	Concentration (kg/l)	Effect on TAN
Deamino-dicarba-oxytocin (D-D-oxytocin)	⌊→Tyr-Ile-Gln-Asn-Asu-Pro-Leu-Gly-NH_2	10^{-4} [a]	E	2×10^{-4}	(—)
Deamino-dicarba-Arg-vasotocin (D-D-Arg-vasotocin)	⌊→Tyr-Ile-Gln-Asn-Asu-Pro-Arg-Gly-NH_2	10^{-4} [a]	E	2×10^{-4}	(—)
Deamino-dicarba-Arg-vasopressin (D-D-Arg-vasopressin)	⌊→Tyr-Phe-Gln-Asn-Asu-Pro-Arg-Gly-NH_2	2×10^{-4}	(—)	2×10^{-4}	(—)

E, excitatory effect. (—), no effect. [a], critical concentration to produce the effect. Asu, α-amino suberic acid.

On the other hand, the microdrop application of D-D-Arg-vasopressin in the same amount did not affect the PON biopotential (Figure 2, C). We conclude from these results that the PON excitation caused by the first 2 peptides is due to the membrane depolarization of this neurone.

In Figure 3, two I–V curves (a, measured in the physiological state, and b, measured 3 min after 2×10^{-4} kg/l D-D-oxytocin application) have been superimposed. In the presence of D-D-oxytocin at this concentration, the PON neuromembrane was remarkably depolarized. In Figure 3A, the two I–V curves have been superimposed using the initial polarization level (just before the transmembrane current application) as the common standard. It seems that the PON neuromembrane resistance may be decreased by D-D-oxytocin. On the other hand, in Figure 3B, the same two I–V curves have been superimposed using the firing level of the neurone as the common standard. The two I–V curves are concordant in the wide range of membrane polarization level. We conclude that the I–V curve measured in the presence of D-D-oxytocin at 2×10^{-4} kg/l is almost identical with that in the physiological state (Figure 3B) and that the depolarization of the neuromembrane, produced by D-D-oxytocin, only appears to cause some decrease of membrane resistance, since the I–V curve of the molluscan giant neurone is not linear[9,10] (Figure 3A).

By bath application the 3 peptides analogous to neurohypophyseal hormones with respect to the excitability of the TAN (tonically autoactive neurone) were examined. These peptides at 2×10^{-4} kg/l had no effect on TAN excitability.

BARKER et al.[11] reported that the neurohypophyseal hormonal peptides, including Arg-vasopressin, oxytocin and Arg-vasotocin, caused membrane depolarization of slowly oscillating giant neurones of *Otala lactea* (Cell 11[12]) and of *Aplysia californica* (R 15[13]), and augmented their bursting pace-maker potential (BPP) activities. Our experimental results with the PON of *Achatina fulica* using synthetic peptides (deamino-dicarba-) analogous to neurohypophyseal hormones were somewhat different than those of GAINER et al., while assuming[4] the PON to correspond to Cell 11 of *Otala lactea*. The critical concentration of D-D-oxytocin and D-D-Arg-vasotocin to produce an excitatory effect on the PON is much higher than that of Arg-vasopressin, oxytocin etc. reported by GAINER et al.

The PON neuromembrane was not sensitive to D-D-Arg-vasopressin at 2×10^{-4} kg/l. The amino acid sequence of D-D-Arg-vasotocin differs only at the second amino acid from that of D-D-Arg-vasopressin. Our results show that the PON neuromembrane can discriminate this structural difference between the two substances. We can say that D-D-oxytocin and D-D-Arg-vasotocin showed a specific excitatory effect on the PON neuromembrane.

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Metabolism of a Biliary Metabolite of Phenacetin and Other Acetanilides by the Intestinal Microflora

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Summary. In vivo, rat intestinal micro-organisms mediate the metabolic hydrolysis of the biliary metabolite (*N-acetyl-p-aminophenyl glucuronide*) *of phenacetin and related compounds.*

In the course of studies on the significance of the intestinal microflora in the metabolism of phenacetin and related compounds in the rat[3,4], evidence has been obtained that *N*-acetyl-*p*-aminophenyl glucuronide is excreted in part in bile and is subsequently metabolized by the intestinal microflora.

Methods and materials. Details of the synthesis of [^{14}C]labelled compounds, chromatographic and radiochemical methods employed are given in our previous publications[3,4]. The biliary metabolite was characterized by co-chromatography with authentic reference compounds[4], autoradiography and by specific chemical and enzyme-degradative procedures[4,5]. Microfloral incubations were carried out by our usual procedure[3]. Conjugates or bile extracts containing conjugates were sterilised by membrane filtration, using sterile Swinnex filter units fitted with white plain filters (Millipore S. A., France).