movements of the foot occurred. We suggest for this behavioral effect the term 'sole detachment'.

The minimum dose of ergometrine or ergotamine producing this effect was 0.4 ml of a 10^{-4} moles/l solution per snail. Lower doses resulted only in hyperactivity; sole detachment did not develop. The 'sole detachment' effect appeared within some min to 1 h depending on the magnitude of the dose. Most snails injected with up to 0.5 ml of a 10^{-3} moles/l solution recovered completely, but larger doses were lethal.

To determine, whether these effects were drug-specific, we investigated behavioral effects of a number of drugs which block DA receptors or otherwise affect synaptic transmission. 4 constant volumes of each of the drugs (0.1, 0.2, 0.4, and 0.8 ml) were injected into the body cavity of snails.

The following substances were used as 10^{-3} moles/l standard solutions dissolved in snail Ringer: haloperidol, chlorpromazine (standard mammalian DA antagonists of the butyrophenone and phenothiazine series, respectively), d-tubocurarine, strychnine (these 2 drugs reportedly eliminate the excitatory responses of snail neurons to DA)[5], methysergide (the most effective antagonist of ergometrine-sensitive serotonin receptors in snails)[6], bromo-LSD (D-2-bromolysergic acid diethylamide), phentolamine, hexamethonium, atropine, tetraethylammonium, morphine, dopamine, serotonin, octopamine, glutamate, acetyl-choline.

Of all the drugs used, only bromo-LSD produced effects which were virtually identical to those of ergometrine and ergotamine. In a previous study we observed similar motor disorders in snails treated with 6-hydroxydopamine[7]. Some of the drugs used in the present study (among them phentolamine, haloperiodole and, notably, serotonin) produced hyperactivity; they could not, however, elicit 'sole detachment'. The results show that hyperactivity alone is not a reliable manifestation of blockade of ergometrine-sensitive DA receptors. Combined with 'sole detachment' and disinhibition, hyperactivity however seems to manifest specific and reproducible behavioral effects of the DA antagonists.

DA is an established neurotransmitter in molluscs capable of exerting both excitatory and inhibitory synaptic effects[6]. Derivatives of lysergic acid such as d-LSD, ergometrine and ergotamine were shown to be the most potent agents affecting DA-sensitive binding of a ^{3}H-labelled ligand to the particulate fraction of *Helix* ganglia[8]. The same substances, as well as 6-hydroxydopamine[9], are the most potent antagonists of hyperpolarizing effects of DA on gastropod neurons[2,3,5,6].

It is obvious from the present results that these agents have a similar exclusive potency in the behavioral effects described here. We conclude that the receptors in question play an important part in the mechanisms controlling locomotion and posture in land pulmonates. This conclusion is in accordance with data from other molluscs which indicate a role of DA in motor control[10-13]. Our results do not necessarily imply that ergometrine-sensitive serotonin receptors are not involved in the above behavioral reactions, although the fact that 6-hydroxydopamine produced the motor disorders while methysergide did not, makes this involvement unlikely. Characteristic and reproducible motor disorders produced by DA antagonists in snails make it possible to use these animals as a simple animal model for screening compounds that affect the specific type of DA receptors.

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1 A.R. Cools and J.M. van Rossum, Life Sci. *27*, 1237 (1980).
2 R.J. Walker, G.N. Woodruff, B. Glaizner, C.B. Sedden and G.A. Kerkut, Comp. Biochem. Physiol. *24*, 455 (1968).
3 G.N. Woodruff, R.J. Walker and G.A. Kerkut, Eur. J. Pharmac, *14*, 77 (1971).
4 K. Meng. Zoll. Jb. *68*, 539 (1958).
5 P. Ascher, J. Physiol. Lond. *225*, 173 (1972).
6 L.D. Leake and R.J. Walker, Invertebrate neuropharmacology. Blackie, Glasgow and London 1980.
7 D.A. Sakharov and J. Salánki, in: Neurotransmitters; comparative aspects, p. 359. Ed. J. Salánki and T.M. Turpaev. Akadémiai Kiadó, Budapest 1980.
8 A.H. Drummond, F. Bucher and I.B. Levitan, Nature, Lond. *272*, 368 (1978).
9 M.S. Berry and G.A. Cottrell, J. Physiol., Lond., *244*, 589 (1975).
10 J. Salánki, Comp. Biochem. Physiol. *8*, 163 (1963).
11 B.M. Twarog and R.A. Cole, Comp. Biochem. Physiol. *43A*, 331 (1972).
12 T. Hidaka, H. Yamaguchi, B.M. Twarog and Y. Muneoka, Gen. Pharmac. *8*, 819 (1977).
13 L. Hiripi, J. Nemcsók, K. Elekes and J. Salánki, Acta biol. Acad. Sci. hung. *28*, 175 (1977).

The pharmacokinetics of VIP in dog and pig

S.J. Mitchell, I.M. Modlin, A.A.J. D'Sa Barros and S.R. Bloom[1]

Department of Medicine, Hammersmith Hospital, London W12 (England), 27 April 1982

Summary. Pure porcine VIP was infused systemically in 4 conscious dogs and systemically and intraportally in 6 anesthetized pigs. At 2.3 pmoles · kg^{-1} · min^{-1} the MCR was 10.7±1.0 ml · kg^{-1} · min^{-1} in dog and 7.6±1.5 (systemic) and 16.5±2.0 (portal) in pig. The $t_{1/2}$'s were 1.0±0.2, 0.85±0.12 and 1.0±0.05 respectively. These values agree with those observed in man. This very high single pass tissue clearance does not suggest a hormonal role for VIP.

VIP has been isolated from the hog small intestine[2] and more recently from the CNS in large quantities[3-5]. It has a wide range of pharmacological actions including powerful vasodilation[2] and potent stimulation of small intestinal secretion[6]. The localization of VIP in fine nerve fibers in the peripheral tissues is in accord with its probable action as a neurotransmitter or neuromodulator[7]. Few physiological stimuli have been shown to cause a significant rise in peripheral plasma VIP but changes are seen after mesenteric ischaemia[8] and vagal stimulation in the calf[9] and pig[10]. A role as a circulating hormone thus cannot be completely excluded. In this study the systemic pharmacokinetics of the dog and pig were investigated and compared with data on the pharmacokinetics of VIP in man[11].

Materials and methods. Four trained conscious mongrel dogs (weight range 12–16 kg) in tight harness restraints were given infusions of VIP or saline in random order on separate days. After an overnight fast the femoral vein was

cannulated for VIP infusion and the femoral artery for blood sampling. Pure natural VIP (kind gift of Prof. V. Mutt) was prepared freshly for each infusion and made up in a solution of 7.5 ml aprotinin (Trasylol 50,000 KIU/ml) and 2.5 ml fresh dog plasma 50 ml 0.9% saline. The infusion rate was 1 ml · min^{-1} and each infusion lasted 60 min. 5 ml of femoral blood was taken at: −30, −15, −5, 0, 15, 30, 45, 60, 60.5, 61, 61.5, 62, 63, 65, 70, 85, 100 and 120 min. Infusate samples were taken prior to, and at completion of the infusion and assayed with the plasma samples. Blood samples were collected into lithium heparinized tubes containing 10% aprotinin (Trasylol 50,000 KIU/ml), centrifuged immediately and stored at −20 °C until assay[12].

6 pigs were studied (weight range: 20-30 kg) after an overnight fast. They were anesthetized with minimal doses of halothane and N_2O. The jugular and portal veins and femoral artery were cannulated. A constant rate pump (5 ml/h) was used to infuse VIP over a 30-min period into either the jugular or portal vein, in random order on different days. Blood and infusate samples were taken from both artery and vein in the same manner as for the dog study. Sampling times were −15, −5, 0, 5, 10, 15, 20, 25, 30, 30.5, 31, 31.5, 32, 32.5, 33 and then at minutely intervals for a further 7 min. Results are given for both systemic and portal infusions in the group of pigs with the SEM for each value. Pharmacokinetic data calculations are based on the plateau principle of Goldstein[13], whereby half life is calculated from the straight line plot of the log post infusion levels, expressed as a percentage of the steady state plateau after subtraction of the basal value. Metabolic clearance is calculated by dividing the infusion rate by the steady state plateau concentration. The apparent distribution space was calculated using the fraction:

$$\frac{\text{MCR} \times {}^1/_2 \text{ life}}{0.693}$$

Results. The basal level of VIP in the dog ranged from 8 to 20 pmoles/l with a mean basal of 15±5 pmoles/l. At VIP infusion doses of 0.8, 1.8, 2.3 and 3.6 pmoles · kg^{-1} · min^{-1} the plateau levels achieved were 86±9, 182±16. 302±15 and 379±15 pmoles/l respectively (fig. 1). Post infusion VIP-values fell to basal level within 10 min. The $t_{1/2}$ was plotted separately for each individual and in each individual the r-value exceeded 0.96 (fig. 2). The $t_{1/2}$-values ranged 0.85-1.3 min with a mean of 1.0±0.04 min. The mean MCR's were 11.1±1.2, 11.5±2.1, 10.7±1.0 and 10.7±0.9 ml · kg^{-1} · min^{-1} for the respective infusion levels. The calculated apparent distribution space varied between 17.8 and 25 ml/kg with a grand mean of 21.1±2.0 ml/kg.

The basal VIP level in the pig was 18±4 pmoles/l, rising to a plateau of 86±6 pmoles/l with the portal venous infusion and to 193±18 pmoles/l (fig. 3) during the systemic infusion, both at 2.3 pmoles · kg^{-1} · min^{-1}. The level of VIP fell rapidly with cessation of infusion, returning to base line values within 6-8 min. The half life following systemic infusion was 0.85±0.12 min while that after portal infusion was 1.0±0.05 min (the individual decay plot r-values being greater than 0.92 in every case). The MCR during systemic infusion was 7.6±1.5 ml · kg^{-1} · min^{-1} and that during portal infusion was 16.5±2.0 ml · kg^{-1} · min with a calculated apparent distribution space of 9.1±4.5 and 23.9±7.2 ml/kg respectively.

Discussion. In both groups of animals the MCR and $t_{1/2}$ of exogenous natural porcine VIP were very similar. The data obtained were consistent with those found during infusion studies in man where the $t_{1/2}$ was 0.96±0.1 min and MCR 8.6±1.3 ml · kg^{-1} · min^{-1} at the infusion level of 3.3 pmoles · kg^{-1} · min^{-1}[14]. In all studies, however, the calculated distribution space was less than plasma volume

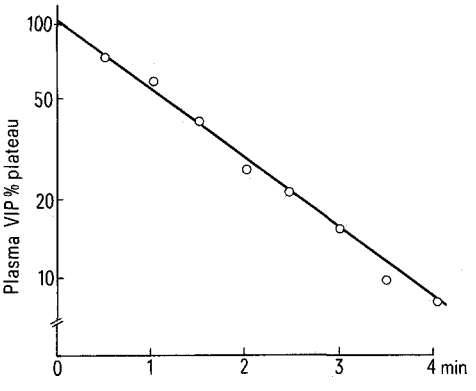

Figure 2. Percent decay curve of VIP increment after stopping VIP infusion in a single representative dog (0.8 pmoles · kg^{-1} · min^{-1}) to illustrate the single order decay kinetics (a similar picture was seen also in the pig studies).

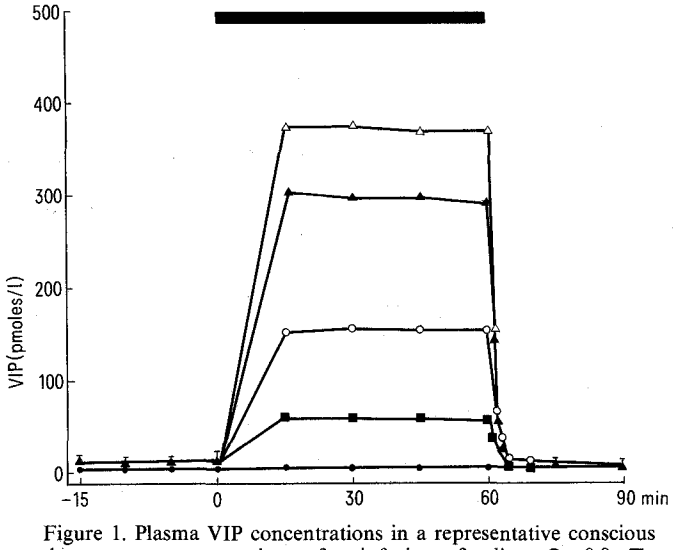

Figure 1. Plasma VIP concentrations in a representative conscious dog on separate occasions after infusion of saline, ●; 0.8, ■; 1.8, ○, 2.3, ▲; and 3.6, △, pmoles · kg^{-1} · min^{-1} into a femoral vein over 60 min (bar).

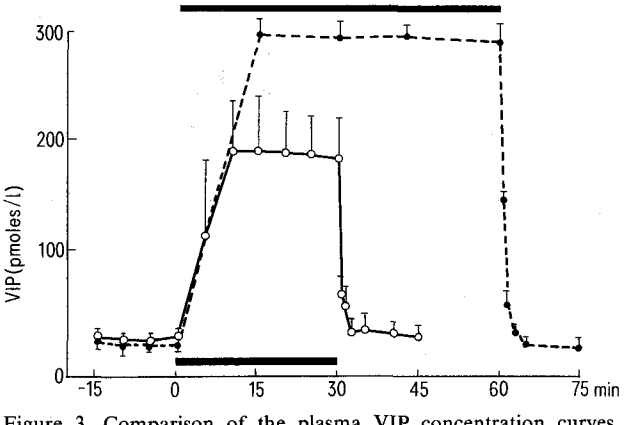

Figure 3. Comparison of the plasma VIP concentration curves following a systemic 2.3 pmoles · kg^{-1} · min^{-1} VIP infusion in 4 dogs (●) and 6 pigs (○), upper and lower bars marking the respective infusion periods.

suggesting the likelihood of incomplete equilibration within the circulation. The very short half life is similar to circulation time suggesting a high extraction rate on passage through the lungs and peripheral tissues.

Despite the identical systemic and portal infusion rate in the anesthetized pig there was a considerable difference in the plateau levels of VIP achieved. The lower systemic levels after portal infusion presumably reflect considerable clearance of exogenous VIP by the liver.

Previous studies, in dogs with completely transposed portal vein and superior vena cava, failed to show a significant hepatic inactivation of VIP[15]. The possible explanations for the different results include altered hepatic function, or development of a portal systemic collateral circulation after transposition. Previous data from an anesthetized pig preparation demonstrated a significant porto-systemic difference in endogenous VIP[16].

The pharmacokinetic data exogenous VIP obtained in dog and pig thus demonstrate rapid clearance and are consistent with a postulated neurotransmitter role.

1 Reprint requests to S.R.B., Francis Fraser Labs, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London, W12OHS.
2 S.I. Said and V. Mutt, Science *169*, 1217 (1970).
3 M.G. Bryant, S.R. Bloom, J.M. Polak, R.H. Albuquerque, I. Modlin and A.G.E. Pearse, Lancet *1*, 991 (1976).
4 S.I. Said and R.N. Rosenberg, Science *192*, 907 (1976).
5 L.I. Larsson, L. Edvinsson, J. Fahrenkrug, R. Hakanson, C.H. Owman and O. Schaffalitsky de Muckadell, Brain Res. *113*, 400 (1976).
6 G.O. Barbezat and M.I. Grossman, Science *174*, 422 (1971).
7 S.R. Bloom and M.G. Bryant, Gut *14*, 823 (1973).
8 I.M. Modlin, S.R. Bloom and S.J. Mitchell, Experientia *34*, 535 (1978).
9 A.V. Edwards, P.M.M. Bircham, S.J. Mitchell and S.R. Bloom, Experientia *34*, 1186 (1978).
10 J. Fahrenkrug, H. Galbo, J.J. Holst and O. Schaffalitzky de Muckadell, J. Physiol, Lond. *280*, 405 (1978).
11 S. Domschke, W. Domschke, S.R. Bloom, P. Mitznegg, S.J. Mitchell, G. Lux and U. Strunz, Gut *19*, 1049 (1978).
12 S.J. Mitchell and S.R. Bloom, Gut *19*, 1943 (1978).
13 A. Goldstein, L. Aronow and S.M. Kalman, Principles of drug action, 2nd edn, p.311. Eds Goldstein, Aronow and Kalman. Wiley, New York 1974.
14 S. Domschke, W. Domschke, S.R. Bloom, P. Mitznegg, S. Mitchell, G. Lux, U. Strunz and L. Demling, in: Gut hormones, p.475. Ed. S.R. Bloom. Churchill Livingstone, Edinburgh 1978.
15 V.T. Strunz, J.H. Walsh, S.R. Bloom, M.R. Thompson and M.I. Grossman, Gastroenterology *73*, 768 (1977).
16 I.M. Modlin, S.J. Mitchell and S.R. Bloom, in: Gut hormones, p.470. Ed. S.R. Bloom. Churchill Livingstone, Edinburgh 1978.

Regional distribution of pancreatic polypeptide cells in the 21-day fetal rat pancreas

Judith L. Shevell and M. R. Schweisthal[1]

Department of Anatomy, School of Medicine, Oral Roberts University, Tulsa (Oklahoma 74171, USA), 30 September 1981

Summary. 21-day fetal rat pancreata were stained with the unlabeled antibody peroxidase-antiperoxidase technique using bovine pancreatic polypeptide as the primary antibody. Total counts of pancreatic polypeptide cells were made over the entire pancreas. It was found that the head region contained the greatest number of pancreatic polypeptide cells with the body next and the tail having the smallest number. The pancreatic polypeptide cells of the body were concentrated in the portion closest to the distal duodenum. This distribution pattern seems to support the suggested role of pancreatic polypeptide on the physiological function of the digestive tract.

Pancreatic polypeptide (PP) is a recently discovered pancreatic hormone found in birds as well as mammals. As has been reported by several workers[2-5] PP has been shown to have an effect on various physiological functions of the digestive tract. Floyd et al.[6] have shown that basal plasma PP levels are significantly elevated in insulin treated diabetic patients compared to healthy subjects. These studies are in agreement with those of Gingerich et al.[5] and correlate with the increased PP cell population that Sundler et al.[7] found in alloxan induced diabetes. Lundquist et al.[8] suggested that PP could have a possible role as a local regulator of glucose induced insulin secretion.

Many studies[7-12] have been done on adult animals to determine the distribution of PP cells. Few if any studies have been performed on neonatal or fetal animals. It was the purpose of this study to examine the PP cell distribution in the 21-day fetal rat pancreas to determine whether there is any substantial difference from the pattern in the adult. If there is such a difference it would merit a study of the neonatal pancreas to determine when the adult pattern is established and possibly by what mechanism(s).

Materials and methods. Intact pancreas, spleen and duodenum were removed from 12 fetal rats of 21 days gestation. 6 preparations were made using 3 procedures: 2 were fixed en bloc and subsequently divided into 3 regions; 2 were divided prior to fixation, and 2 were pinned en bloc to lead lined redwood blocks. Pins stabilizing the head region were placed in the duodenum, those for the tail pierced the spleen, additional pins were placed in the mesentery. Tissues and blocks were submerged in Bouin's fixative for 20 min, then pins and blocks were removed and the straight, flattened tissue bloc returned to the fixative. The latter was the method of choice. Regardless of method used all tissues were fixed for 16 h in Bouin's fluid.

Following routine histological procedures the tissues were embedded flat in paraplast and serially sectioned at 4 μm. The slides were stained with the unlabeled antibody peroxidase-antiperoxidase technique[13]. A 1:100 dilution of bovine pancreatic polypeptide (BPP) was used as the primary antibody (lot number 615-R110-146-16, graciously

Regional distribution of pancreatic polypeptide cells of 21-day fetal rat pancreata*

Number of PP cells per region Region	Fetus I	Fetus II
Head	1134	1030
Body	639	612
Tail	112	390

*As determined by qualitative methods over 10 slides for each of 2 pancreata.