1 Acknowledgments. This work was supported in part by grant No. 8461 d/82 of the National Research Council Argentina and by Research Contract No. 2816/R1/R2 of the International Atomic Energy Agency. The authors wish to acknowledge the valuable technical assistance of Miss Ofelia Chiesa and Miss Patricia Mandalunis.

2 Neuman, W.F., Fleming, R.W., Dounce, A.L., Carlson, A.B., O'Leary, J., and Mulryan, B., J. biol. Chem. *173* (1948) 737.
3 Neuman, W.F., Neuman, M.W., and Mulryan, B.J., J. biol. Chem. *175* (1948) 705.
4 Adams, N., and Spoor, N.L., Phys. Med. Biol. *19* (1974) 460.
5 Neuman, M.W., and Neuman, W.F., J. biol. Chem. *175* (1948) 711.
6 Rowland, R.E., and Farnham, J.E., Hlth Phys. *17* (1969) 139.
7 Stevens, W., Bruenger, F.W., Atherton, D.R., Smith, J.M., and Taylor, G.N., Radiation Res. *83* (1980) 109.
8 Picer, M., and Strohol, P., Analyt. chim. Acta *40* (1968) 131.
9 Lanfranchi, H.E., and de Rey, B.M., J. dent. Res. *61* (1982) 615.
10 Itoiz, M.E., Carranza, F.A. Jr, and Cabrini, R.L., J. Periodont. *34* (1963) 305.
11 Weibel, E.R., and Elías, H., in: Quantitative Methods in Morphology, p. 3. Eds E.R. Weibel and H. Elías, Springer Berlin/Heidelberg/New York 1967.
12 López Otero, R., Carranza, F.A. Jr, and Cabrini, R.L., J. periodont. Res. *8* (1973) 327.
13 Guglielmotti, M.B., and Ubios, A.M., J. dent. Res. *61* (1981) 615.
14 Haley, D.D., Lab. Invest. *46* (1982) 168.
15 de Rey, B.M., Lanfranchi, H.E., and Cabrini, R.L., Envir. Res. (1983) in press.

0014-4754/84/050474-03$1.50 + 0.20/0

The effect of ether, pentobarbitone sodium and fentanyl on blood gases, acid-base balance and hematological parameters in the rat

J. Komarek*,[1]

Ciba-Geigy Ltd, CH-4002 Basle (Switzerland), 20 December 1982

Summary. Blood gases, acid-base balance and hematological parameters (RBC, PCV and Hb) were measured in adult rats of both sexes. The use of ether and fentanyl had a very little effect on the blood gases and acid-base balance. The induction of pentobarbitone anesthesia, however, was followed by a significant increase in PCO_2 and TCO_2, while the pH value decreased.

Many investigations have been performed in an attempt to provide normal values for the blood gases and the acid-base balance of the rat[2-8], but there have been surprisingly few carefully-controlled studies comparing the effects of the anesthetics and mode of blood sampling on these parameters. It has been reported that the blood values of anesthetized rats may not accurately reflect the acid-base status in conscious ones[9]. Therefore we performed experiments to compare the effects of anesthesia and analgesia on hematological parameters, blood gases and acid-base balance with the influence of manual restraint.

Materials and methods. Studies were made using adult rats of both sexes (Tif: RAIf, Ciba-Geigy) weighing between 220 and 300 g, which were kept under S.P.F. conditions with access to food and water ad libitum. The animals were fasted overnight, from about 16.00 h until the experiment was started around 08.00 h the next morning. Either manual restraint or anesthesia was employed while postorbital puncture (retroorbital venous plexus) was carried out to remove blood from each animal; it was drawn anaerobically before the anesthetic (ether or pentobarbitone sodium) or analgesic agent (fentanyl) was administered (pre-treatment values), and a 2nd sample taken as the rat succumbed to anesthesia or analgesia (treatment values), i.e. when drowsiness became apparent. The time point at which the 2nd blood sample was taken was identical for all 3 drugs. To prevent hypothermia the rats were placed in an insulated container. Rectal temperature was taken in some animals randomly selected and was found to be within normal limits (normothermia) for this strain of rat.

Ether anesthesia was induced by placing the rat ($n=30$) in a covered glass jar containing a pad of cotton wool soaked in diethylether (Aether ad narcosin, Siegfried, Switzerland). Pentobarbitone sodium (Nembutal, Abbott Laboratories, USA) was administered by i.p. injection using a dose of 5 mg/100 g b.wt ($n=20$).

Analgesia ($n=20$) was produced by i.p. injection of 0.1 ml fentanyl solution/100 g b.wt (Fentanyl Janssen, Belgium). Each ml of the solution containing 0.05 mg of the analgesic and sedative fentanyl.

Because of the limited blood sampling that can be done in the rat without the risk of interfering with the hematological parameters, a separate study was made on 15 control animals to check its influence on the values being measured.

Blood gases and acid-base balance were analyzed immediately after sampling by an electrode system (micromethod, Blood Gas Analyzer, Corning, USA). Two buffer solutions and 2 gas mixtures were used for calibration. All hematological measurements (RBC, PCV and Hb) were made with the Coulter Counter S-Plus (Coulter Electronics, Inc., Florida, USA) which was calibrated in the standard manner.

The t-test[10] for differences between paired observations was used to analyse these data, and significance was accepted at the 1% level.

Results. The results are expressed as means and SD. Effects of anesthesia and analgesia: Table 1 shows the values obtained for blood gases, acid-base balance and hematological parameters measured immediately before and during anesthesia or analgesia. The use of ether and fentanyl had very little effect on the blood gases and acid-base balance; except for the increase ($p < 0.01$) in PO_2 caused by ether, there were no significant differences between conscious (before anesthesia) and anesthetized animals. In the present experiment, pentobarbitone narcosis was followed by an increase in PCO_2 ($p < 0.01$), TCO_2 ($p < 0.01$) and bicarbonate level ($[HCO_3^-]$) in the blood, while the

pH-value and PO_2 were significantly ($p < 0.01$) reduced. As shown in table 1, the values for RBC, PCV and Hb were altered by all 3 drugs. The results indicate clearly that the use of ether and fentanyl produced only moderate decreases in the hematological parameters, whereas the administration of pentobarbitone led to more pronounced changes: the erythrocyte count showed a decrease of 19%, the hematocrit fell by 20%, and there was a reduction of the hemoglobin content by 20%.

Control animals. Blood was drawn to determine the influence of repeated sampling on the hematological values measured. The results are shown in table 2. The mean values should be considered as normal reference values. Except for the significant increase in PO_2, the statistical analysis by Student's t-test did not demonstrate any significant differences between the effects of the first and second blood samplings.

Discussion. The removal of blood from the rat usually imposes a certain degree of stress. During toxicological investigations in our laboratory, a number of blood sampling methods have been used to compare the effects of restraint by means of narcosis with ether, pentobarbitone sodium or fentanyl (analgesia) against those of manual restraint.

Brun-Pascaud et al.[11] stated recently that when rats were anesthetized with pentobarbitone (30 mg/kg b.wt, i.p.) abnormal values were found: the arterial blood gases and pH values were significantly different from those in other groups of unanesthetized rats (increased $PaCO_2$ and decreased pHa). Similar effects on blood gases (PCO_2, TCO_2) and acid-base status (pH, $[HCO_3^-]$) were also seen in our experiments with pentobarbitone sodium: the induction of anesthesia caused ventilatory depression accompanied by respiratory acidosis - increased PCO_2 and TCO_2 and a decrease in pH are found in association with an increase in the blood bicarbonate $[HCO_3^-]$ level. These observations are in contrast to the results of Libermann et al.[5], who reported no difference between anesthetized and conscious rats, but are in agreement with the findings of other authors[9,11,12]. Compared with the results in the dog, the effects of pentobarbitone sodium in the rat are similar with respect to the fall in PO_2: Priano et al.[13] noted an arterial PO_2 decrease of 28 mm Hg after i.v. administration of 30 mg pentobarbitone/kg b.wt; Steiner and Cavlin[14] found PaO_2 to be reduced during pentobarbitone anesthesia (25 mg/kg b.wt, i.v.) by 10 mm Hg. The reduction of PO_2 in the blood recorded in our experiments (table 1) is the result of central respiratory depression by the barbiturate, a side effect which is well known[15].

No significant changes in PCO_2, TCO_2, pH and $[HCO_3^-]$ were observed in animals which had received anesthetic doses of ether or the neuroleptanalgesic agent fentanyl (table 1). The significant increase in PO_2 found in the group of animals anesthetized by ether cannot be explained by ventilatory stimulation via ether-induced release of catecholamines alone; the catecholamine release may, however, lead to an improvement of the homogeneity of V°/Q°-relation throughout the lung parenchyma, by influence on the bronchomuscular tone.

Furthermore, we found that the use of pentobarbitone led to significant and very marked changes in the hematological values measured (RBC, PCV and Hb), whereas the administration of ether and fentanyl had only a moderate effect. The notable change in the erythrocyte count (decrease of 19%) seen here in pentobarbitone-anesthetized rats has also been observed by Graca and Garst[16] in dogs anesthetized with pentobarbitone sodium: they reported a decrease in the erythrocytes of 16–17%. Srivastava et al.[17] also described a similar reduction in the RBC (12–20%) in dogs after the administration of pentobarbitone sodium. The observed changes in the hematocrit (decrease of 20%) produced by the i.p. injection of pentobarbitone in rats (table 1) are similar to those found by other workers in the dog[17]; they may be caused by a pentobarbitone-induced engorgement of the spleen[18,19], or by a rapid rise in extracellular fluid volume after anesthesia with pentobarbitone, which is usually accompanied by a decrease in the hematocrit[20].

In conscious, manually-restrained control rats, repeated blood sampling did not alter (except for an increase in PO_2–$p < 0.01$) the values measured significantly. The blood pH of manually-restrained rats, represented in table 1 as pre-treatment values of conscious rats before anesthesia, and of the control animals in table 2 are practically identical with the normal values reported for conscious rats by different authors[2,8].

The absence of any detectable alteration in the pH suggests that the method of manual restraint employed in this experiment did not lead to any increased blood acidity, such as has been described in conscious rats by Dawson et al.[21], and by Upton and Morgan[22]. The elevated level of PO_2 recorded in the manually-restrained rats at the 2nd blood sampling was probably due to hyperventilation caused by the stress imposed by this procedure.

Our observations suggest that when anesthesia cannot be avoided during blood collection in the rat, light ether anesthesia or analgesia with fentanyl can be recommended as an acceptable alternative to restrain the animals.

Table 2. Control rats (n = 15). The effect of repeated blod sampling on blood gases, acid-base balance and hematological values in conscious manually-restrained animals

Parameter	1st blood sampling	2nd blood sampling	%	p
PO_2 (kPa)	4.64 ± 0.34	5.07 ± 0.50	+9.3	*
PCO_2 (kPa)	5.21 ± 0.77	5.30 ± 0.61	+1.7	NS
TCO_2 (mmol/l)	28.2 ± 3.0	30.0 ± 2.7	+6.4	NS
pH	7.45 ± 0.09	7.47 ± 0.04	+0.3	NS
$[HCO_3^-]$ (mmol/l)	27.0 ± 3.1	28.8 ± 2.6	+6.7	NS
RBC (T/L)	8.6 ± 0.5	8.4 ± 0.6	−2.3	NS
PCV (1)	0.48 ± 0.03	0.47 ± 0.03	−2.1	NS
Hb (mmol/l)	10.3 ± 0.5	9.9 ± 0.6	−3.9	NS

Means ± SD, NS = not significant, * significant at 1% level. Blood from the retroorbital venous plexus.

Table 1. The effect of anesthesia and analgesia on blood gases, acid-base balance and hematological parameters in the rat

Parameter	Ether anesthesia (n = 30)				Pentobarbitone anesthesia (n = 20)				Fentanyl analgesia (n = 20)			
	Before	During	%	p	Before	During	%	p	Before	During	%	p
PO_2 (kPa)	5.01 ± 0.41	6.12 ± 1.20	+22.2	*	5.31 ± 0.77	4.61 ± 0.53	−15,2	*	5.20 ± 0.59	5.09 ± 0.49	−2.1	NS
PCO_2 (kPa)	4.45 ± 0.50	4.29 ± 0.59	− 3.6	NS	5.17 ± 0.45	7.44 ± 1.24	+43.9	*	5.82 ± 0.37	5.58 ± 0.46	−4.1	NS
TCO_2 (mmol/l)	24.9 ± 2.6	24.9 ± 2.1	± 0	NS	28.1 ± 2.5	30.7 ± 4.0	+ 9.3	*	30.0 ± 3.3	31.0 ± 2.7	+3.3	NS
pH	7.47 ± 0.07	7.48 ± 0.04	± 0	NS	7.45 ± 0.03	7.32 ± 0.10	− 1.7	*	7.42 ± 0.06	7.46 ± 0.05	+0.5	NS
$[HCO_3^-]$ mmol/l	24.0 ± 2.5	23.9 ± 2.0	0.4	NS	26.9 ± 2.4	28.9 ± 4.0	+ 7.4	NS	28.6 ± 3.3	29.7 ± 2.7	+3.8	NS
RBC (T/L)	8.5 ± 0.8	8.0 ± 0.8	− 5.9	NS	8.3 ± 0.6	6.7 ± 0.3	−19.3	*	7.4 ± 0.7	7.0 ± 0.4	−5.4	*
PCV (1)	0.48 ± 0.03	0.46 ± 0.03	− 4.2	*	0.46 ± 0.02	0.37 ± 0.02	−19.6	*	0.43 ± 0.03	0.40 ± 0.02	−7.0	*
Hb (mmol/l)	10.3 ± 0.6	9.7 ± 0.5	− 5.8	*	10.2 ± 0.4	8.2 ± 0.3	−19.6	*	9.4 ± 0.7	8.8 ± 0.4	−6.4	*

Means ± SD, NS = not significant, * significant at 1% level. Blood from the retroorbital venous plexus.

* Present address: Tierärztliche Klinik, Schlachthofplatz 12, D-8560 Lauf/BRD.
1 Acknowledgments. The author gratefully acknowledges the excellent technical assistance of I. Bolliger, J. Heller, S. Küng, B. Müller, B. Schmidt and U. Völkle.
2 Altland, P.H., Brubach, H.F., Parker, M.C., and Highman, B., Am. J. Physiol. *212* (1967) 142.
3 Kaczmarczyk, G., and Reinhardt, H.W., Lab. Anim. Sci. *25* (1975) 184.
4 Lewis, L.D., Ponten, U., and Siesjö, B.K., Respir. Physiol. *19* (1973) 312.
5 Libermann, I.M., Capano, A., Gonzales, F., Brazzuna, H., Garcia, H., and Gelabert, A.G., Lab. Anim. Sci. *23* (1973) 862.
6 Marder, J., and Bar-Ilan, A., Physiol. Zool. *48* (1975) 282.
7 Pepelko, W.E., and Dixon, G.A., J. appl. Physiol. *38* (1975) 518.
8 Simons, W.B., Kahn, F.H., and Goze, L.B., Fedn Proc. *25* (1966) 1274.
9 Bar-Ilan, A., and Marder, J., Pflügers Arch. *384* (1980) 93.
10 Bailey, N.T.J., Statistical Methods in Biology. The English Universities Press, London 1959.
11 Brun-Pascaud, M., Gaudebout, C., Blayo, M.C., and Pocidalo, J.J., Respir. Physiol. *48* (1982) 45.
12 Ledsome, J.R., Linden, R.J., and Norman, J., J. Physiol. *212* (1971) 611.
13 Priano, L.L., Traber, D.L., and Wilson, R.D., J. Pharmac. exp. Ther. *165* (1969) 126.
14 Steiner, S.H., and Calvin, J.R., J. thorac. cardiovasc. Surg. *54* (1967) 592.
15 Sharpless, S.K., The barbiturates, in: The Pharmacological Basis of Therapeutics, 4th edn, p.105. Eds L.S. Goodman and A. Gilman. MacMillan, London 1970.
16 Graca, J.G., and Garst, E.L., Anaesthesiology *18* (1957) 461.
17 Srivasta, S., Khanna, D.N., and Shukla, R.C., Indian J. Physiol. Pharmac. *9* (1966) 63.
18 Gilmore, J.P., Am. J. Physiol. *209* (1965) 404.
19 Nash, C.B., Davis, F., and Woodbury, R.A., Am. J. Physiol. *185* (1956) 107.
20 Rampton, D.S., and Ramsey, D.J., J. Physiol. *237* (1974) 521.
21 Dawson, C.A., Nadel, E.R., and Horvath, S.M., J. appl. Physiol. *30* (1971) 322.
22 Upton, P.K., and Morgan, D.J., Lab. Anim. *9* (1975) 85.

0014-4754/84/050476-03$1.50 + 0.20/0

Potentiating action of hexoprenaline on ^{14}C-aminopyrine uptake by isolated rat parietal cells

S. Maśliński and H.-J. Ruoff

Pharmakologisches Institut der Universität Tübingen, Wilhelmstr. 56, D-7400 Tübingen (Federal Republic of Germany),
26 June 1983

Summary. Hexoprenaline potentiated the ^{14}C-aminopyrine uptake (a reliable index of H^+ generation) of isolated rat gastric cells stimulated by 10^{-6} –10^{-4} mol/l carbachol, and inhibited that in response to 10^{-4} mol/l histamine without and in the presence of propranolol. It is concluded that hexoprenaline acts as a partial agonist on parietal cell H_2-receptors and that β-adrenoceptor activation may functionally modulate gastric acid secretion.

The presence of histamine, acetylcholine and gastrin-receptor sites on parietal cells is well documented[2]. Secretagogues interact and potentiate each other in vivo[3] and in vitro[4]. Histamine exerts its action by stimulation of H_2-receptor sensitive adenylate cyclase which then initiates the intracellular steps of H^+ production[5]. It was previously shown that β-adrenergic stimulation also activates gastric mucosal adenylate cyclase[6-9]. Moreover, we found that the selective β_2-receptor agonist hexoprenaline stimulated the ^{14}C-aminopyrine (^{14}C-AP) uptake of isolated gastric cells[9], a reaction which reflects acid production inside the parietal cell[10]. The present investigation studied the interaction of hexoprenaline on the ^{14}C-AP uptake in response to histamine or cholinergic stimulation by carbachol.

Experimental. Cell isolation and ^{14}C-AP uptake measurements have been described previously in detail[9,11]. All experiments with histamine were performed in the presence of 10^{-3} mol/l isobutylmehtylxanthine (IBMX) and 10^{-3} mol/l Ca^{2+}. The carbachol experiments were done without IBMX and in the presence of 2×10^{-3} mol/l Ca^{2+}. The results were evaluated by the t-test for paired data.

Results. The effect of hexoprenaline, alone and with 10^{-4} mol/l histamine, a maximal effective concentration, on ^{14}C-AP uptake is shown in figure 1. Hexoprenaline stimulated the basal uptake by 100% ($p < 0.001$), 10^{-5} mol/l being the maximal effective concentration[9]. The adrenergic β-receptor antagonist propranolol reduced the hexoprenaline stimulation and 10^{-7} mol/l were almost sufficient to abolish any response. The histamine H_2-receptor antagonist cimetidine also exerted inhibitory potencies; however, concentrations higher than 10^{-7} mol/l were required.

The histamine-stimulated ^{14}C-AP uptake was much more pronounced than that following hexoprenaline, and was significantly reduced by 16.5–30.5% in the presence of 10^{-6}–10^{-4} mol/l hexoprenaline. Propranolol (10^{-7} mol/l) reduced the histamine stimulation by 12% (n.s.), but did not reverse the action of 10^{-4} mol/l hexoprenaline, which decreased the histamine effect by 38% ($p < 0.01$) in the presence of the adrenergic β-blocker. When lower, i.e., threshold concentrations of histamine (10^{-6} mol/l) were used, the results were less clear and in some experiments even potentiation of ^{14}C-AP uptake was observed.

For optimal cholinergic stimulation the cell medium had to be IBMX free and contained 2×10^{-3} mol/l Ca^{2+}. Carbachol stimulated the AP uptake in a concentration-dependent manner, maximally by 85% (10^{-4} mol/l). Without IBMX, hexoprenaline alone evoked no response. However, the β_2-receptor agonist increased significantly the ^{14}C-AP uptake due to carbachol, which now stimulated the uptake maximally by 134%. It seems that this effect represents true potentiation, since the values are higher than the calculated sum of responses to both compounds.

Discussion. The data confirm and extend our previous findings with β-receptor agonists[9] and demonstrate a potentiating interaction between hexoprenaline and carbachol on ^{14}C-AP accumulation of isolated rat gastric cells. They are partly contradictory to the general view that adrenergic stimulation solely inhi-